



**MICROBIAL REDUCTION OF NITRATE IN IRRIGATED SOIL
AFTER WASTEWATER APPLICATION**

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ABSTRACT

Nitrogen is an essential nutrient for plant growth and is routinely added in the form of fertilizer or via irrigation water for optimal crop production. Nitrogen losses through denitrification have an environmental and economical impact.

The end product of topsoil denitrification is likely to be N_2O rather than N_2 , because in topsoil, N_2O readily diffuses into the atmosphere before further reduction to N_2 . This increases concerns over N_2O emissions into the atmosphere, since N_2O is a greenhouse gas and also causes depletion of the ozone layer. Furthermore, if the N added with the fertilization practice is being reduced to gaseous forms through the denitrification process, this has a negative impact on crop production and is not economically valuable. It is therefore important to estimate the N-gaseous losses through denitrification in the irrigated topsoil. It has been common practice since the 1950s to irrigate arid and semi-arid agricultural land with treated wastewater since this optimizes water use in water-limited environments and adds a considerable amount of N to the soil. Although using wastewater for irrigation purposes represents a good source of water in arid and semi-arid regions and also provides a considerable amount of some essential nutrients (e.g. N) which are required for plant growth, it also contains high levels of soluble sodium and exhibits a high Biological Oxygen Demand (BOD_5), which can potentially increase gaseous N losses. Therefore, it is particularly important to study N losses through topsoil denitrification in topsoil of wastewater-irrigated arid and semi-arid land.

Intact and repacked soil cores from two sites located in the Thessaloniki plain in Northern Greece were used in order to study the effect of irrigation water quality, soil texture and structure, soil moisture and oxidizable organic carbon on total topsoil denitrification rates. These two sites were separated by a distance of 10 km. The first site was the Galicos River Wastewater Treatment project, where soil cores of clay loam and sandy silt loam were obtained from two areas, each irrigated either with secondary wastewater or fresh water (well water) supplied by a furrow for four years. The second site consisted of fields located in the Axios Delta region, irrigated

naturally by the flooding of the Axios River. Clay, clay loam and sandy loam soil cores were collected from these fields.

The microbial reduction of nitrate in the top layer of irrigated soils was studied in laboratory topsoil incubations using the acetylene inhibition method. The use of acetylene to inhibit further microbial reduction of nitrous oxide to dinitrogen allows estimates of total denitrification to be obtained by gas chromatography analysis of nitrous oxide.

Incubations of repacked soil cores from the Galicos River site which were amended with carbon in the form of glucose were found to have a total denitrification rate ranging from 1.41 to 2603 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹. Unamended repacked soil had total denitrification rates ranging from 1.1 to 2.3 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹. Total denitrification rates measured in incubations of intact soil cores from the same field site and amended with carbon were found to range from 1.21 to 92.90 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹. Unamended intact soil cores had total denitrification rates ranging from 1.08 to 1.33 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹. The differences in total denitrification rates in amended and unamended repacked soil cores were statistically significant and indicate that available carbon may be a factor limiting topsoil denitrification in repacked soil cores from this site. In addition, the differences in total denitrification rates between wastewater and well water irrigated soil cores from this site were statistically significant and indicate that soil quality after irrigation was probably a factor regulating the topsoil denitrification in repacked and intact soil cores. A crust layer produced on the surface of repacked and intact soil cores had no significant effect on total denitrification rates.

Total denitrification rates in soil core incubations from the Axios Delta site ranged from 0.71 to 178 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹ under field capacity moisture status and natural carbon concentrations. N₂O gaseous emissions in the soil core incubations from these fields were found to increase exponentially in the initial phase, after which N₂O concentrations decreased, probably due to the consumption of acetylene (C₂H₂) by micro-organisms allowing the reduction of N₂O to N₂ to take place. This exponential increase in N₂O emission indicates that a decrease in O₂ diffusion into

soil aggregates and into denitrification sites is the main factor limiting topsoil denitrification in the Axios field site. Results from the investigation of the effect of soil texture on denitrification rates disagreed with the general view that higher denitrification rates are expected to occur in fine-textured soils.

In order to evaluate the accuracy of the N_2O measurements, a ^{15}N balance for the soil core incubations from the Galicos River site was calculated. This balance indicated that the ^{15}N unrecovered from soil analysis at the end of the incubation time averaged 55 and 36 % of the nitrogen applied to repacked soil cores from wastewater and well water irrigated plots, respectively, and that unrecovered ^{15}N accounted for an average of 14.43 and 12.07 % of the total nitrogen applied to intact soil cores irrigated with wastewater and well water, respectively. Of these values, emitted N_2O gas accounted for an average of 35 and 28 % of the nitrogen applied to repacked soil cores from wastewater and well water irrigated plots, respectively, and for an average of 1.02 and 0.07 % of the total nitrogen applied to intact soil cores irrigated with wastewater and well water, respectively. The unaccounted nitrogen losses at the end of incubation constituted an average of 20 and 8 % of the total nitrogen added to repacked cores under wastewater and well water irrigated plots, respectively, and an average of 13.4 and 12 % of the total nitrogen added to the intact cores. These unaccounted ^{15}N losses indicate that there are additional sinks for added nitrogen besides those measured in the incubations, and that part of the N_2O escaped measurement because of its further reduction to N_2 when incomplete acetylene inhibition of N_2O reductase occurred.

DECLARATION

I hereby declare that this thesis is my own composition and the work reported was carried out by myself, except where I have stated. The work presented here has not been submitted in any previous application for a higher degree.

Khereya Ahmed Ben Farag

DEDICATION

To my loving mother and in memory of my dear father

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SYMBOLS USED

Symbol	Description	Unit	Reference
EC	Electrical Conductivity The reciprocal of the electrical resistivity. The resistivity is the resistance in ohms of a conductor. It is expressed in reciprocal ohms, mmho cm ⁻¹ or dS m ⁻¹ .	mmho cm ⁻¹ or dS m ⁻¹	Richards, 1954
TDS	Total Dissolved Salts The total salts concentration in soil in relation to potential of salinity effects on plant growth. TDS (mg l ⁻¹) = EC (dS m ⁻¹) × 640	(mg l ⁻¹)	Bohn <i>et al</i> , 1985
SAR	Sodium Adsorption Ratio It is a ratio for soil extracts and irrigation waters used to express the relative activity of sodium ions in exchange reactions with soil. $SAR = \frac{Na}{\sqrt{\frac{(Ca + Mg)}{2}}}$ where the ionic concentrations are expressed in (mmol _e l ⁻¹).	mmol _e l ⁻¹	Richards, 1954
SAR _{adj}	Adjusted Sodium Adsorption Ratio $SAR_{adj} = \frac{Na}{\sqrt{\frac{(Ca + Mg)}{2}}} [1 + (8.4 - pH_c)]$ where pH _c = the theoretical pH value (All cations in mmol _e l ⁻¹)	mmol _e l ⁻¹	(Ayers, 1977)
ESP	Exchangeable Sodium Percentage The degree of saturation of the soil exchange complex with sodium. ESP = (Exchangeable sodium / Cation exchange capacity) × 100	%	Richards, 1954.

where
the ionic concentrations are expressed in millimol of
charge per 100 g of soil (mmol_c 100 g⁻¹ soil)

RSC	Residual Sodium Carbonate	mmol_c l⁻¹	Eaton, 1950; Richards, 1954.
	$RSC = (CO_3^{--} + HCO_3^-) - (Ca^{++} + Mg^{++})$		
	<p>where</p> <p>ionic concentrations are expressed in millimol of charge per litre (mmol_c l⁻¹).</p>		
LR	Leaching Requirement	Fraction or %	Rowell, 1994; Richards, 1954
	<p>The fraction of the water entering the soil that must pass through the root zone in order to prevent soil salinity from exceeding a specified value. It is a ratio of the equivalent depth of the drainage water to the depth of irrigation water (D_{dw}/D_{iw}).</p> $LR = EC_{iw} / EC_{dw} = D_{dw}/D_{iw}$		
TEC	Total Electrolyte Concentration	mmol_c l⁻¹	Sumner, 1993
	<p>Is the sum of the positive and negative charged ions that are measured in solution.</p>		
IR	Infiltration Rate	cm h⁻¹	Dupriez and Leener, 1992
	<p>Downward movement of irrigation water or rainfall into the soil surface.</p>		
HC	Hydraulic Conductivity	cm h⁻¹	Dupriez and Leener, 1992
	<p>The ability of a soil to conduct water within its volume, usually in the downward direction. The value measured includes the effects of parameters such as tortuosity of the flow path, pore size distribution of the soil and viscosity and density of the liquid.</p>		

BOD₅	Biological Oxygen Demand	mg l⁻¹	Pescod, 1992
	The amount of oxygen, in mg l ⁻¹ , used by microorganisms for the mineralization under aerobic conditions of the organic matter present in the water, after a time period of 5 days and at a temperature of 20°C.		
COD	Chemical Oxygen Demand	mg l⁻¹	Pescod, 1992
	The amount of oxygen, in mg l ⁻¹ , necessary for chemical oxidation of the organic material to carbon dioxide and water.		
TOC	Total organic carbon	µg C g⁻¹ dry soil	Nelson and Sommers, 1982
	Is the sum of the carbon in the soil organic fraction, which consists of the cells of microorganisms, plant and animal residues at various stages of decomposition, stable "humus" synthesized from residues and highly carbonized compounds such as charcoal, graphite and coal.		
WSOC	Water-soluble organic carbon	µg C g⁻¹ dry soil	Nelson and Sommers, 1982
	Is the soluble fraction from soil organic carbon and usually measured in soil extracts.		
E_h	Redox Potential	Volt (V)	Patrick <i>et al</i> , 1996
	Redox potential is the quantitative measure of the electron availability or potential in soil oxidation-reduction reactions.		

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1 INTRODUCTION

Nitrogen is an essential constituent of all cells, making up 1 to 10 % by weight in plants, and up to 20 to 30 % in animals (Campbell, 1983).

Nitrogen is usually taken up by plants as the simple inorganic ions: nitrate (NO_3^-) and ammonium (NH_4^+). These nutrients reach the plant roots either by mass flow, diffusion or by both methods (Nye and Tinker, 1977). However, some plants are able to use dinitrogen (N_2) through symbiotic associations with nitrogen fixing bacteria. The bulk of nitrogen in plants occurs in proteins, nucleic acids and amino acids. Nitrogen is also an important building block in the structure of nucleic acids, chlorophyll, adenine and a number of the plant growth regulators. With increases in nitrogen supply, there are increases in both soluble amino compounds and proteins. However, nitrogen deficiency results in plants with yellow or pale green leaves with small cells and thick walls, and the leaves may be harsh and fibrous (Wild and Jones, 1988).

Nitrate ions (NO_3^-) are present in soil from either direct addition as fertilizer, through irrigation water such as wastewater irrigation or through nitrification, the microbial oxidation of the ammonium ion (NH_4^+) (Brady, 1974). Once in the soil, NO_3^- can be taken up by plant roots, leached (transported in solution down the soil profile), assimilated by micro-organisms (immobilisation) or microbially reduced.

Gaseous forms of N are lost by the processes of nitrification and denitrification. Denitrification is the microbial reduction of nitrogen oxides, NO_3^- and nitrite (NO_2^-) to the nitrogenous gases, nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) under low oxygen (O_2) concentrations (Bremner and Shaw, 1958a; Tiedje, 1982; Granli and Bøckman, 1994). The main denitrifying genera in soil include *Bacillus*, *Paracoccus*, *Pseudomonas* and *Spirillum* (Ingraham, 1981; Focht, 1982). Denitrifiers are generally facultative anaerobes, which energetically prefer to use O_2 as the electron acceptor in respiration (Focht, 1982; Tiedje, 1982). However, at low O_2 concentrations, denitrifiers can utilise NO_3^- and NO_2^- as alternative terminal electron acceptors (Tiedje, 1982).

Nitrogen can be lost from terrestrial ecosystems as nitrous oxide (N_2O) emissions. Denitrification and nitrification processes are the most important soil sources of N_2O . Nitrous oxide gas is emitted naturally by biological processes from soil and water bodies as a consequence of N turnover (Bouwman, 1990a; Riley and Vitousek, 1995). Production of N_2O from terrestrial ecosystems occurs continuously in small amounts and this is the main source of N_2O in the atmosphere (Freney *et al.*, 1979). However, the amount emitted can be enhanced by changes in land use, agricultural and industrial activities, and the combustion of solid wastes and fossil fuels.

Many anthropogenic sources of N_2O have been identified, such as sewage water, animal manure, ground water, crop residues, soil organic matter, industrial processes and fossil fuel. In addition, other sources are proposed, e.g. automobile emissions and climatic change (Fowler, 1990; Cofer *et al.*, 1991; Russell *et al.*, 1991; Thiemans and Trogler, 1991; Khalil and Rasmussen, 1992; Weier *et al.*, 1994; Lessard *et al.*, 1996; Chang *et al.*, 1998; Flessa *et al.*, 1998; Fey *et al.*, 1999; Akiyama *et al.*, 2000; Scott *et al.*, 2000; Sommer and Møller, 2000). These anthropogenic sources have led to an increase in atmospheric N_2O concentrations over time.

Measurements of atmospheric N_2O concentrations are very important because of the impact of N_2O on the environment and have improved since the 1990s due to the advancement of technology (Khalil and Rasmussen, 1992; Nevison, 1997; Mosier, 1998). Mosier (1998) reported that about 100 Tg of N (Tg terra gramme = 10^{12} g) is moved around the globe as ammonia (NH_3), nitric oxide (NO) and nitrous oxide (N_2O) annually.

Besides contributing to a loss of N from the soil, emissions of N gases cause environmental problems, especially N_2O , which acts as both a greenhouse gas and in destruction of the ozone layer (Bouwman, 1990b). Therefore, recent environmental concern has focused on the possible damaging effect of the gaseous products of denitrification on the stratospheric ozone layer and subsequently on plant and animal life on Earth (Crutzen, 1981, Bouwman, 1990b), and also on the contribution of

N₂O to global warming. Nitrous oxide is an efficient absorber of infra-red radiation, and enhances the 'greenhouse' effect in the atmosphere (Crutzen, 1981; Yoshinari, 1990; Groffman, 1995). It has an atmospheric lifetime of approximately 100 to 200 years, and therefore changes in concentration have a long-term effect (Bouwman, 1990a; Fowler, 1990; Liu and Reiners, 1999).

Whereas N₂ is an inert and harmless product, NO, although at low atmospheric concentration, participates in atmospheric processes that affect ozone concentrations (Delwiche, 1981). Nitrous oxide is oxidised by a photochemical reaction in the stratosphere into the gases nitrogen dioxide (NO₂) and NO (Crutzen, 1981). These gases catalyse the destruction of ozone and limit its abundance in the stratosphere (Crutzen, 1981; Bouwman, 1990a). Nitric oxide derived from N₂O may play a positive role in the stratosphere, reacting with degradation products from volatile chlorinated carbon compounds and diminishing their destructive effect on ozone. However, as emissions of chlorofluorocarbons decrease, the detrimental effect of N₂O on stratospheric ozone may dominate any positive effect (Granli and Bøckman, 1994).

Global losses of N₂O from fertilizer have been estimated to account for 1 to 4 % of the N applied as fertilizer, with an annual mean of 1.5 Tg N (Fowler, 1990). Currently, atmospheric N₂O is increasing at a rate of about 0.2 to 0.3 % year⁻¹ (Fowler, 1990; Granli and Bøckman, 1994), and it is estimated that N₂O has contributed to about 5 % of the 'greenhouse' warming since the 18th century (Bouwman, 1990a). Plants require nitrogen for growth, which in conventional agriculture is supplied by nitrogen fertilizers (Lynch, 1988); therefore losses of N from agricultural land have also given rise to economic concerns.

Secondary wastewater irrigation of soils has been widely practised since the middle of the 19th century in arid and semi-arid regions, where evaporation exceeds precipitation (Pescod, 1992). Large quantities of wastewater effluent are used for irrigation in southern and western USA, Mexico, Australia, dry regions of Africa, Asia and Europe (Feigin *et al.*, 1991). Examples include Libya (Africa), Greece (Europe) and China (Asia). In China, sewage use in agriculture has developed

rapidly since 1958 and now over 1.33 million hectares are irrigated with sewage effluent (Pescod, 1992).

Wastewater irrigation is a valuable resource for increasing agricultural production. This is because it provides valuable water resources and also has a high nutritional value, thus reducing or eliminating the requirements for commercial fertilizers (Pescod, 1992). Irrigation with wastewater effluent results in the addition of considerable amounts of nitrogen to the soil (Bielorai *et al.*, 1984). However, its availability and high biological oxygen demand (BOD_5) increases the potential of irrigated soil for N-gaseous losses through denitrification. In addition, the high sodium content in wastewater has the ability to disperse soil, resulting in reduced hydraulic conductivity of the soil profile and a reduction in the infiltration rate of water and leads to poor aeration. These changes in soil properties are likely to reduce gas exchange within soil pores and the diffusion of O_2 into the soil, resulting in an increase in denitrification rates. Studies at the University of Edinburgh have shown that the N_2O emission rate is strongly dependent on the water filled pore space of soil (Dobbie *et al.*, 1999).

The addition of nitrogen to the soil through irrigation with wastewater affects nitrogen uptake by the crop, the level of available nitrogen in the soil, gaseous nitrogen losses and N leaching below the rooting depth. These differences are widely affected by climatic conditions, soil and wastewater properties, cropping, fertilizer and irrigation management. Irrigation management using reclaimed effluent depends upon the chemical composition of the wastewater, in addition to other factors such as fertilisation, drainage and soil properties.

The objective of this work was to investigate the microbial reduction of nitrate (denitrification) in topsoil irrigated with different water qualities (wastewater, fresh water and river water) and the factors that control this process, taking into account the soil moisture, structure and soil texture variability. The initial work focused on; (a) identification of changes in soil properties under wastewater irrigation compared with fresh water irrigation and (b) inducing the crust formation on the repacked and intact soil cores from the Galicos River site exhibiting a range of exchangeable

sodium percentage (ESP) and electrical conductivity (EC) levels using a rainfall simulator drop impact. Fine-textured soils do not generally drain well; consequently, they retain large quantities of water for long periods, which enhances development of anaerobic conditions. The wetting of light structurally unstable soils containing high Na concentrations using low salinity water such as rainwater or good quality irrigation water, may result in crust formation (Sumner, 1993). This is followed by a reduction in the infiltration rate and we hypothesise that this also enhances the development of anaerobic conditions and leads to an increase in microbial NO_3^- reduction.

Topsoil cores from two sites were incubated in closed systems to determine the fate of NO_3^- -N in irrigated soils of different ESP/EC combinations, textures and structures and irrigated with different water sources. Topsoil cores from these sites were incubated at different soil moisture status. Oxidizable organic carbon was added to the soil cores to determine its role on topsoil denitrification. The information obtained from this work will be useful in indicating the best agricultural practice under which soil nitrogen loss can be reduced in these irrigated soils.

1.1 Thesis structure

Chapter 1 provides an introduction to the thesis. Chapter 2 gives a general literature review. The third chapter gives an outline of the general materials and methods used. Chapter 4 explores the effect of secondary municipal wastewater irrigation compared to well water irrigation on soil properties over four years in the Galicos River Wastewater Treatment Project in the Thessaloniki plain in northern Greece. Chapter 5 evaluates the effect of irrigation either with wastewater or well water over four years on denitrification losses when nitrate was applied to these soils. Chapter 6 examines the influence of oxidizable carbon supply on denitrification losses of soils irrigated either with wastewater or well water. Chapter 7 examines the denitrification potential of different soil textures from the Axios Delta fields in The Thessaloniki plain in northern Greece. Chapter 8 concludes the major findings of this study. Chapter 9 points out areas for further research and outlines some recommendations.

2 LITERATURE REVIEW

2.1 Wastewater reuse

2.1.1 Wastewater treatments

Wastewater is commonly classified as domestic, industrial or agricultural according to the source. About 80 % of wastewater comes from domestic sources (Feigin *et al.*, 1991). Wastewater for irrigation use is treated in order to protect the public, both from consumption of contaminated crops and from direct exposure to the applied effluent (Feigin *et al.*, 1991). Wastewater treatment processes are classified as primary, secondary, tertiary and quaternary (Shuval, 1977). Primary treatment, is the removal of coarse solids and other large materials that are often found in raw wastewater. The main objective of secondary treatment is to remove the residual organic and suspended solids. The tertiary treatment consists of the removal of suspended solids and nutrients remaining in the secondary clarified effluent by flocculation. The quaternary or advanced treatment aims at upgrading the effluent to the level of fresh potable water and employs techniques such as ultrafiltration or distillation by which any undesirable constituent, including excess salinity, can be removed from the water (Feigin *et al.*, 1991; Pescod, 1992). In the USA, the reuse of sewage effluent released $0.76 \text{ Mm}^3 \text{ day}^{-1}$ of fresh water (Pescod, 1992). The suitability of domestic wastewater for irrigation depends mainly on the chemical composition and the degree of treatment (Thomas and Law, 1968).

The simplest secondary treatment can be provided by oxidation ponds in which oxygen is produced by natural surface aeration, and by algae through photosynthesis. This oxygen is used by bacteria for decomposition of organic matter and the nutrients released are consumed by the algae (Feigin *et al.*, 1991). The wastewater effluents from municipal plants that have had the secondary treatment carried out on them are widely used for irrigation. Quality standards of reclaimed wastewater are usually designed to obtain maximum benefit from irrigation water. Besides crop yield and its quality, soil fertility, groundwater properties and environmental quality are affected by the quality of irrigation water.

Typical properties of secondary municipal effluents are presented in Table 2.1. These may influence the response of soils to irrigation with secondary wastewater (Thomas and Law, 1977; Idelovitch, 1978; Asano *et al.*, 1985).

Table 2.1 Characteristics of secondary municipal effluents (Thomas and Law, 1977; Idelovitch, 1978; Asano *et al.*, 1985).

Constituent	Range	
	Min	Max
Total solids	400	1200
Total suspended solids	10	100
Total dissolved solids	400	1100
pH	7.8	8.1
BOD ₅	10	80
COD	30	160
Total nitrogen	10	50
Nitrate-nitrogen	0	10
Ammonium-nitrogen	1	40
Total phosphorus	6	17
Chlorides	40	200
Alkalinity (Calcium carbonate)	200	700
Sodium	50	250
Potassium	10	40
Calcium	20	120
Magnesium	10	50
Boron	0	1
SAR	4.5	7.9

*All units are in mg l⁻¹, excluding pH and SAR (mmol_c l⁻¹)

2.1.2 Wastewater irrigation impact on irrigated soil properties

Many studies have focused on the effects of wastewater irrigation on soil properties (Bole *et al.*, 1981; Hayes *et al.*, 1990; Feigin *et al.*, 1991; Abo-Ghobar, 1993; Al-Jaloud *et al.*, 1993; Bajwa *et al.*, 1993; Friedel *et al.*, 2000; Panoras *et al.*, 2001a, 2001b). These studies have shown that wastewater reuse can cause both beneficial and detrimental changes in the irrigated soil system. Wastewater causes a build-up

of inorganic soluble salts, especially sodium chloride, and substances that are toxic to plants and animals, such as boron, chloride and molybdenum. The concentration of other trace elements may also increase.

The electrical conductivity (EC) and sodium adsorption ratio (SAR) of irrigation water were found to be the most useful criteria for irrigation water classification (Richards, 1954; Rhoades *et al.*, 1992). Residual sodium carbonate (RSC) and adjusted SAR (SAR_{adj}) are also important. Eaton (1950) indicated that determination of bicarbonate (HCO_3^-) ion concentrations in irrigation water is important. This is because of its ability to precipitate calcium and magnesium in the form of carbonate salts. As a result, Na^+ ions become predominant on cation exchange sites of irrigated soil and sodic soil results. This relationship can be expressed by calculating the concept of residual sodium carbonate (RSC) to evaluate which waters have high carbonate and consider the detrimental hazards of using high-carbonate levels of water for irrigation which could produce a RSC value of more than $2.5 \text{ mmol}_e \text{ l}^{-1}$.

Wastewater reuse may be responsible for salinisation of soils through the migration of salts in water, accumulation through evaporation and deposition, especially in arid and semi-arid regions. The increase in soil salinity is consistent with the salt loading for the different effluent irrigation qualities (Hayes *et al.*, 1990; Al-Jaloud *et al.*, 1993; Falkiner and Smith, 1997). Al-Jaloud *et al.* (1993) found a strong positive correlation between salinity of irrigation water and the resultant soil salinity ($r = 0.98$). As a result, the application of 15-20 % of excess water was recommended to meet leaching requirements to maintain soil salinity within acceptable limits for optimal production of sorghum (*Sorghum vulgare* Pers, a Sudan cv. Beef Builder) and maize (*Zea mays* L., a US cv. Atlantic Yellow Hybrid Sheet Corn) crops. In addition, the higher dry matter production observed from sorghum crop compared with maize receiving the same quantity of irrigation water was because sorghum is more salt tolerant than corn at all growth stages (Al-Jaloud *et al.*, 1993). Falkiner and Smith (1997) observed that the electrical conductivity measured in a saturated paste extract of surface soil (0 - 5cm depth) increased from 1 to 3 dS m^{-1} after four years irrigation using wastewater of 0.79 dS m^{-1} electrical conductivity.

Pnarasimharao and Ynarasimharao (1992) reported that the salinity of effluent with low sodium hazard and no residual sodium carbonate has not created any soil problems and can be used for irrigation of rice and cotton. However, it is not suitable for irrigation of crops which are less tolerant to salinity e.g. tobacco and chilli. Kumar *et al.* (1998) found that mixed industrial effluent increased the salinity of soil and shallow surface water resources.

In evaluating the suitability of waters for irrigation, an important consideration is the extent to which the exchangeable sodium percentage (ESP) will increase in the soil. Therefore, any suitable evaluation of the potential sodicity hazard of irrigation water must take into account the Sodium Adsorption Ratio (SAR) of the soil water. Experiments already conducted on the effect of total electrolyte concentration and sodium adsorption ratio of the percolating solution on soil sodicity can be seen in Appendix 1. The SAR provides a reliable estimate of the ESP in soils. Richards (1954) used SAR in place of ESP for diagnosing sodicity problems. The potential hazard of irrigation water with a high sodium adsorption ratio on soil physical properties has been widely investigated (McNeal and Coleman, 1966; Frenkel *et al.*, 1978; Oster and Schroer, 1979; Hayes *et al.*, 1990; Russell *et al.*, 1991; Pescod, 1992; Falkiner and Smith, 1997; Balks *et al.*, 1998). Experiments already conducted on the effect of exchangeable sodium percentage (ESP) on N₂O emission from illitic soil aggregates can be seen in Appendix 3.

High wastewater sodium content leads to an increase in the ESP of irrigated soil (Paliwal and Grandhi, 1976; Al-Jaloud *et al.*, 1993). This is due to the ability of sodium present at high concentrations in wastewater to displace divalent ions in the cation exchange sites. Balks *et al.* (1998) reported that the irrigation of *Pinus radiata* and *Eucalyptus grandis* with either a treated sewage effluent of EC 0.78 dS m⁻¹, SAR 5 or bore water of EC 0.67 dS m⁻¹, SAR 3.1 over 5 seasons of irrigation increased the exchangeable sodium percent of irrigated soil from <2.0 % throughout the soil profile to >25 % at some depths within the 0.6 m of soil surface. As a result, the increase in soil sodicity resulted in an increased tendency of the soil to disperse, while the magnitude of the measured decrease in saturated hydraulic conductivity (K_{sat}) of irrigated soil was not sufficiently large enough to cause a restriction to soil

water flow. They concluded that the increase in ESP of the irrigated soil did not seem likely to affect its continued use for effluent irrigation in the short term. However, Abu-Sharar *et al.* (1987) reported that the K_{sat} of a Fallbrook Haplic soil column of sandy loam soil texture, with unstable aggregates and a high portion of macropores, and containing 5.4 g C g^{-1} soil, was reduced to zero after percolation of deionized water of SAR 10 and an electrolyte concentration of $2.5 \text{ mol}_c \text{ l}^{-1}$, compared with a K_{sat} of 1.93 cm h^{-1} in a Pachappa Haplic soil column of loam soil texture with highly stable aggregates and a high portion of micropores, and containing 4.6 g C kg^{-1} soil. The reduction in K_{sat} of the Fallbrook soil might be attributed to the reduction of large conducting pores as a result of aggregate slaking.

Maintaining stable soil structure and adequate water infiltration rates is important for all cultivated soils. The amounts of various soil constituents such as organic matter, iron and aluminium oxides, percent and mineralogy of clay fraction, and calcium carbonate play an important detrimental role on physical and chemical characteristics of irrigated soils when using water of high sodium content (McNeal and Coleman, 1966; Frenkel *et al.*, 1978; Oster and Schroer, 1979; Agassi *et al.*, 1981; Shainberg *et al.*, 1981; Goldberg *et al.*, 1988).

In a study by Agassi *et al.* (1981), the reduction in infiltration rate (IR) occurred at ESP values of 1 in a non-calcareous (Netanya) and of 6.4 in a calcareous (Nahal-Oz) soil which contained 13 % CaCO_3 when both soils were irrigated using water with a low total-electrolyte concentration. McNeal and Coleman (1966) reported that decreasing electrolyte concentrations and increasing the SAR of the percolating solution led to a decrease in hydraulic conductivity (HC) of irrigated soils. The decreases in HC were large for soils high in 2:1 layer-silicates, particularly soils containing mostly montmorillonite, compared with the HC of soils high in kaolinite and sesquioxides that were less sensitive to increasing sodium adsorption ratio of applied water. However, a soil containing considerable amorphous material was much more stable. They concluded that even for soils high in 2:1 layer-silicates and containing mostly montmorillonite, the large reduction in HC is not expected even at 15 % or more exchangeable sodium, when salt concentrations of the applied water are more than $3 \text{ mmol}_c \text{ l}^{-1}$. Frenkel *et al.* (1978) observed that when applying water

with a high sodium concentration, the reduction in HC on soil columns packed with montmorillonite was higher than in soil columns packed with kaolinite or vermiculite. They reported that the plugging of pores by dispersed clay particles was a major cause of reduced soil hydraulic conductivity for surface soils irrigated with sodic waters. In addition, the sensitivity to excessive exchangeable sodium and low electrolyte concentration increased with clay content and bulk density. Moreover, although the reduction in HC of kaolinitic soil was lower than that of the montmorillonitic soil, its HC was reduced even more drastically (even at an ESP of 10) when leached with water of low salinity (Frenkel *et al.*, 1978).

Oster and Schroer (1979) found that high concentrations of the HCO_3^- ion in irrigation water led to calcium precipitation as CaCO_3 in soil columns. As a result, the sodium adsorption ratio was increased and resulted in IR reduction of irrigated soils. However, the IR of irrigated soils was increased from 2 to 28 mm h^{-1} by increasing the electrolyte concentration in the irrigation water. This is because an increase in soil salinity reduces the potential of Na^+ to deflocculate soil particles. Bole *et al.* (1981) observed that high HCO_3^- ion concentrations (580 mg l^{-1}) in municipal wastewater with SAR 3.2 increased the SAR of irrigated soil solution because of its effect on calcium precipitation.

Most of the suspended solids in raw wastewater are removed by proper treatment. However, some suspended organic and inorganic solids may remain in the effluents. The concentration of organic matter in treated domestic effluent is about 150 g m^{-3} (Noy and Feinmesser, 1977). When these suspended solids are applied to the soil through irrigation water, they can clog capillary pores, mainly in the upper most layer of heavy soils. As a result, the soil infiltration rate (IR) decreases. Suspended solids may also close capillaries deeper in the soil profile and lead to a decrease in soil permeability.

Vinten *et al.* (1983a, b) observed that the flow rate in coarse sand soil columns, packed at a bulk density of 1.30 mg m^{-3} and leached with unfiltered secondary sewage effluent (EC 1.97 dS m^{-1} , pH 7.6, SAR 5.7, Na^+ 250 g m^{-3} , total suspended solids 98 g m^{-3} , chemical oxygen demand 0.316 kg m^{-3} and volatile suspended solids

84 g m⁻³) decreased by no more than 25 %. In addition, passing unfiltered effluent through the coarse sand resulted in retention of 43 % of the total suspended solids by the soil. The high flow rate observed in these columns is attributable to the coarseness of the porous medium, which can retain much of the suspended solids but without much clogging of pores. However, a high reduction in flow rate was observed in silt loam soil columns (23.1% clay, 0.95 % organic matter), packed at a bulk density of 1.30 mg m⁻³ and leached with secondary sewage effluent filtered through Whatman no. 92 paper (total suspended solids 38 g m⁻³ and volatile suspended solids 38 g m⁻³). The flow declined to 20 % of its initial value after 400 mm of filtered effluent had been added, and only a very small fraction of suspended solids was observed in the leachate. This might be due to the accumulation of solids at the soil surface.

Richards (1954) suggested that a SAR value above 10 and an ESP value of 15 % are the critical levels above which soil structure is damaged. As a result of the loss of structure, soil permeability problems occur. However, Crescimanno *et al.* (1995) reported that significant soil degradation could occur even within a 2 – 5 % ESP range at a low cationic concentration.

Raindrop impact was found to decrease the soil infiltration rate (IR). This is due to surface crust formation (Abo-Ghobar, 1993; Bajwa *et al.*, 1993). Bajwa *et al.* (1993) observed that the IR of a bare sandy loam soil, after irrigation over six years with saline sodic wastewater (EC 0.82 dS m⁻¹, SAR_{adj} 3.70, TDS 525 mg l⁻¹) with high residual alkalinity and NaCl concentration, decreased progressively as the amount of water applied by a stationary spray system increased. The effect was observed at two application rates of 25 and 100 mm h⁻¹, but especially under the higher application rate. The IR was even lower when irrigation was carried out with sodic clean water (EC 0.41 dS m⁻¹, SAR_{adj} 1.60, TDS 450 mg l⁻¹) with residual alkalinity. The reduction in IR under saline sodic wastewater irrigation might be due to the clogging of soil pores because of accumulation of suspended solids at the soil surface.

Similarly, Abo-Ghobar (1993) conducted an experiment whereby a bare sandy loam soil of electrical conductivity (EC) ranging from 0.21 to 0.28 dS m⁻¹, ESP 1.4 - 3.6 % was treated with three different irrigation water qualities over the course of 6 years. The water treatment applied consisted of a sodic treated wastewater (EC 1.16 dS m⁻¹, SAR 12.20, RSC 9.5 mmol_c l⁻¹) with high NaHCO₃ concentration, a saline sodic treated wastewater (EC 2.90 dS m⁻¹, SAR 29.40, RSC 9.5 mmol_c l⁻¹) with high NaHCO₃ and NaCl concentrations and good quality canal water (EC 0.21 dS m⁻¹, SAR 0.39) with no RSC. It was observed that the exchangeable sodium percentage (ESP) of the treated soils increased by 32.1 % and 39.2% when irrigated with sodic and saline sodic wastewater, respectively (compared to the results obtained under irrigation with good quality canal water). Therefore, since an increase in ESP augments the potential for clay dispersal, the infiltration rate (IR) of soil irrigated with the sodic and the saline sodic treated wastewater significantly decreased during Abo-Ghobar's study (1993).

Studies of the soil contamination with pathogens and heavy metals due to irrigation with wastewater have been conducted since the beginning of the 1980s (Banin *et al.*, 1981; Bole *et al.*, 1981; Russell *et al.*, 1991; Levy and Kearney, 1999). These studies reported that the appropriate wastewater treatments could provide a high level of health protection.

The reuse of wastewater not only conserves valuable water resources, but also takes advantage of the nutrients contained in sewage to grow crops. The increase in soil nitrogen concentration was consistent with the increase in nitrogen in treated wastewater (Al-Jaloud *et al.*, 1993; Hussain *et al.*, 1996). High water salinity can neutralise the beneficial effects of nutrients in wastewater. Al-Jaloud *et al.* (1993) observed that the green biomass of corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) crops irrigated with saline wastewater (up to 2330 mg l⁻¹ TDS) was increased. This might be attributed to the high level of nitrogen (up to 80 mg l⁻¹) in irrigation water. Hussain *et al.* (1996) reported that the high grain yield and nitrogen use efficiency of wheat (*Triticum aestivum* L.) crop can be achieved by low application rates of nitrogen fertilizer under irrigation with treated effluent containing nitrogen in the range of 20 mg l⁻¹ and above. Moreover, the wastewater

irrigation containing 40 mg N l⁻¹ would be sufficient to obtain optimum grain yield without any additional fertilizer nitrogen.

Hayes *et al.* (1990) observed a significant increase in soil NO₃⁻-N concentration after 16 months from irrigation of turfgrass using secondary municipal wastewater (NO₃⁻-N 1-7.5 mg l⁻¹, NH₄⁺-N 0- 28.6 mg l⁻¹) compared to soil NO₃⁻-N concentration under potable irrigation of NO₃⁻-N 1 - 5 mg l⁻¹ and NH₄⁺-N 0 - 1.5 mg l⁻¹. This increase in soil NO₃⁻-N concentration is attributable to the high total N concentration in the treated wastewater source.

The effect of wastewater irrigation on soil organic matter status has been widely investigated. Some of these studies have shown positive results (Burns and Rawitz, 1981; Friedel *et al.*, 2000). Burns and Rawitz (1981) observed that subsequent drying improved the drainage of a heavy soil during irrigation with effluent due to the effect of organic matter additions. Friedel *et al.* (2000) reported an increase in the total organic carbon contents (TOC) at 0–15 cm depth in vertisols whose clay ranged from 38.8 to 55.8 %, TOC 1.1-2.7 %, pH 7.4-8.1, EC 0.98-4.48 dS m⁻¹, after irrigation over 25, 65 and 80 years with municipal wastewater of slightly alkaline pH (8.4) with medium to high salinity hazard. In addition, the increase in the total organic carbon content was significantly related to the duration of irrigation and increased 2.5 fold after 80 years of irrigation. This might be due to the high irrigation water requirements of alfalfa, and also vertisols need a high quantity of water to reach field capacity (the moisture content of soil in the field two or three days after a thorough wetting of the soil profile by rain or irrigation water). Another possibility is that the increase in total organic carbon content is due to the long-term irrigation under Friedel's study.

Hayes *et al.* (1990) reported a decrease in pH from 7.9 to 7.4 in Typic Haplargid after irrigation over 16 months either with secondary treated municipal wastewater (pH 7.0 - 9.5, EC 0.65 - 0.91 dS m⁻¹, SAR 3.2 - 4.1, NO₃⁻-N 1.0 - 7.5 mg l⁻¹ and NH₄⁺-N 0.0 - 28.6 mg l⁻¹) or potable well water (pH 7.5 - 8.4, EC 0.2 - 0.3 dS m⁻¹, SAR 0.7 - 1.6, NO₃⁻-N 1.0 - 5.0 mg l⁻¹ and NH₄⁺-N 0.0 - 1.5 mg l⁻¹). This is probably attributed to the nitrification effect of added nitrogen in Hayes's study.

2.2 Soil N cycle

2.2.1 The soil N cycle as part of a global cycle

Nitrogen occurs in the four recognized spheres of the earth: the lithosphere, atmosphere, hydrosphere and biosphere. The bulk of the N is found in the lithosphere (98 %) and most of the remainder occurs in the atmosphere, where dinitrogen (N_2) comprises about 78 % of the gases. However, unlike in the other spheres, the N in the biosphere is in a constant state of flux, which is commonly described as the N cycle (Figure 2.1). This links the nitrogen in all four spheres and any given N atom moves from one form to the other in a completely irregular fashion (Stevenson, 1982). Plant available forms of nitrogen usually only occur at a very low concentration in soil (Woodmansee *et al.*, 1981). The amounts of NO_3^- and NH_4^+ in the soil profile are controlled by a number of complex, simultaneous and often opposing processes occurring in soils, dominantly mediated by micro-organisms, and known as the soil nitrogen cycle (Figure 2.2).

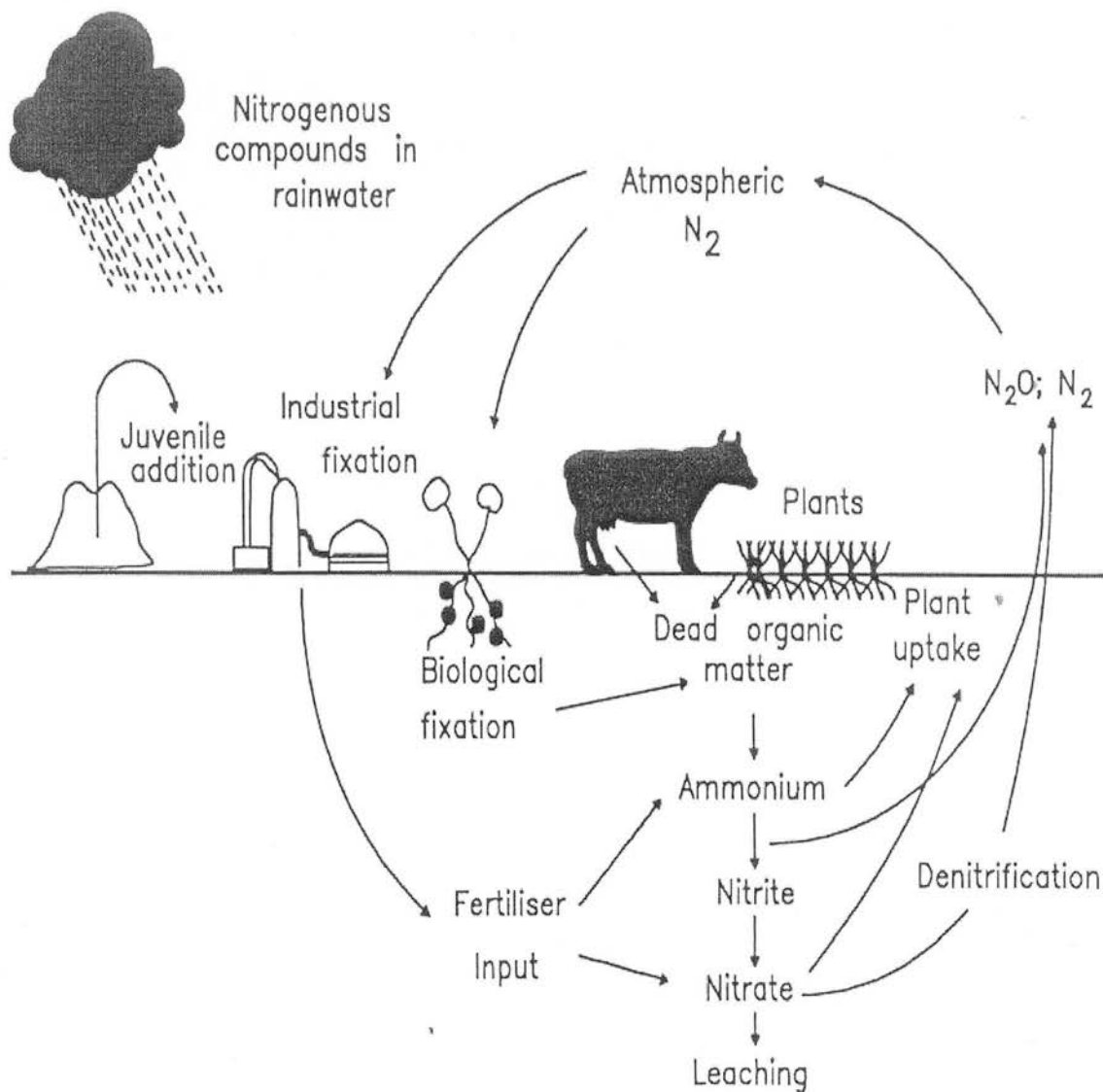


Figure 2.1 The global N cycle, based on Haynes (1986)

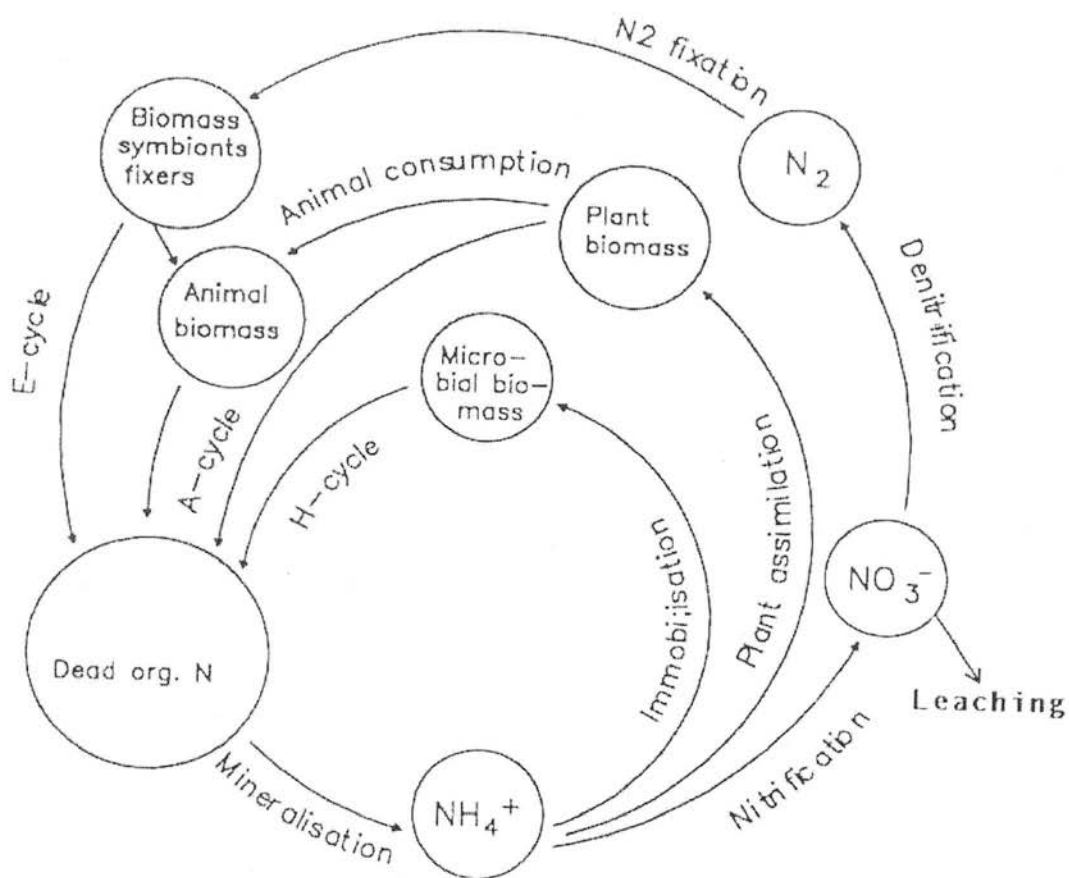


Figure 2.2 The soil N cycle, divided into its three subcycles: the elemental (E), the autotrophic (A) and the heterotrophic (H), adapted from Jansson and Persson (1982).

2.2.1.1 Nitrogen transformations in the soil

The mineral nitrogen that is the direct nitrogen source for all non-fixing plant species accounts for about 1% of the total nitrogen content in the upper layer of soils (Woodmansee *et al.*, 1981). The overwhelming proportion of the nitrogen is present in the form of organic compounds. There is continuous cycling of nitrogen within the soil. N is added to the soil via the biological fixation of atmospheric N₂ gas by N-fixing micro-organisms (either free-living or in symbiotic association with plants) or by industrial fixation during fertilizer production and as a by-product of other industrial processes. Living and dead organic matter also provide actively-cycled reservoirs of N. Soil losses occur through leaching of mineral nitrogen, plant uptake followed by harvesting and through denitrification (Figure 2.1).

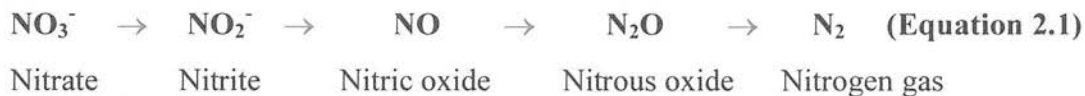
Energy flows through the N cycle, beginning with the absorption of solar energy by plants and some micro-organisms in photosynthesis (Jansson and Persson, 1982), and there are strong links between this flow of energy and the cycling of N. Since most processes of the soil N cycle are microbially mediated, the soil N cycle is heavily dependent on the C flow through the soil, and the interaction between the two cycles often controls the availability of N for plants.

2.2.1.2 Integration of soil nitrogen subcycles within the universal nitrogen cycle

The overall nitrogen cycle can be simplified by dividing it into three interdependent subcycles (Jansson and Persson, 1982) as shown in Figure 2.2. The elemental subcycle (E) comprises the pathway which allows nitrogen from the atmosphere to enter the soil nitrogen system, via N-fixation, and ultimately return to the atmosphere via denitrification. The autotrophic subcycle (A) comprises the incorporation of inorganic nitrogen into plants, and subsequent build up of organic substances through the process of photosynthesis, followed by eventual degradation upon death. The third subcycle, the heterotrophic subcycle (H), involves heterotrophic micro-organisms which make up the microbial biomass. It is within this subcycle that the processes of mineralisation and immobilisation occur. These processes, linked with the uptake, assimilation and decomposition of plants, produce a continuous turnover of nitrogen and other elements in the soil.

2.2.2 Denitrification within the soil N cycle

Denitrification is an irreversible process and as such represents a loss of nitrogen from the biosphere to the atmosphere in the last step of the N-cycle. Biological denitrification is the microbial reduction of nitrogenous oxides to gaseous N:



Denitrification consists of four sequential reactions. The first reaction, reduction of NO_3^- to NO_2^- , is catalysed by the enzyme nitrate reductase. The second reaction, reduction of NO_2^- to NO , is catalysed by nitrite reductase. The third reaction, reduction of NO to N_2O , is catalysed by nitric oxide reductase. The final reaction, reduction of N_2O to N_2 , is catalysed by nitrous oxide reductase.

Biological denitrification requires a decomposable organic substrate, usually low O_2 concentration, the presence of N oxides, and suitable temperature and pH conditions. The denitrification process is carried out by bacteria that use N oxides as terminal electron acceptors in the place of molecular O_2 (Focht, 1982; Powlson, 1988). Fungi have also been shown to be capable of reducing NO_2^- and NO_3^- anaerobically (Shoun *et al.*, 1992).

The production of nitrogenous gases has been observed between redox potential (E_h) values of 0.4 and 0.1 V (Bailey and Beauchamp, 1973). Reduction of NO_3^- and NO_2^- has been reported to lead to a decrease in redox potential (Bailey and Beauchamp, 1973). As NO_2^- is reduced at lower E_h (0.2 V) than NO_3^- (0.35 V), NO_3^- is preferentially reduced at low O_2 concentrations (Bailey and Beauchamp, 1973; Stolzy and Fluhler, 1978).

Denitrifiers are among the most successful physiological groups of micro-organisms in nature, constituting approximately 20% of the bacterial population capable of anoxic growth, and 1 to 5% of the total heterotrophic population that can be isolated (Tiedje *et al.*, 1982). Denitrifying micro-organisms are found in virtually every

physiological and taxonomic group of prokaryotes (Ingraham, 1981), and include diverse genera such as *Pseudomonas*, *Alcaligenes* and *Azospirillum* (Beauchamp *et al.*, 1989). Most denitrifiers of importance in soil are heterotrophic, facultative anaerobes (Tiedje, 1982). However, autotrophic denitrifiers such as *Paracoccus denitrificans* and *Alcaligenes eutrophus* (capable of growing on hydrogen), the sulphur-oxidising *Thiobacillus denitrificans* (Beauchamp *et al.*, 1989) and denitrifying fungi (Shoun *et al.*, 1992) have also been isolated. Autotrophic denitrifiers obtain their energy by using NO_3^- to oxidise inorganic compounds, e.g. reduced sulphur (S^{2-}) compounds and ferrous (Fe^{2+}) iron (Granli and Bøckman, 1994).

Denitrifiers have a unique set of enzymes that conserve energy from the reduction of NO_3^- and NO_2^- by electron transport phosphorylation (Tiedje, 1982). However, not all denitrifying bacteria have the enzymes necessary to carry out the full pathway (Ingraham, 1981).

Denitrifiers are generally facultative anaerobes which prefer energetically to use O_2 as the electron acceptor in respiration (Focht, 1982). However, at low O_2 concentrations denitrifiers can utilise NO_3^- and NO_2^- as alternative terminal electron acceptors (Tiedje, 1982). The specific regulatory control of the nitrogen oxide reductases is not clearly understood, but it appears that there are always small quantities of enzymes present (Focht, 1982), which may explain the immediate, but small, amount of denitrifying activity observed upon a shift from oxic to anoxic conditions.

Chemodenitrification is a non-biological process (Granli and Bøckman, 1994). It is thought to be most important in acidic soils (Brady, 1974; Tiedje, 1982). N_2O can be generated by the reaction of nitrite and hydroxylamine, both of which are produced during the reduction of nitrate to ammonium and the oxidation of ammonium to nitrite (Focht, 1982). Chemodenitrification has been observed as being responsible for <10 to 20% of the total N_2O flux in laboratory incubations of low pH soils (Parkin *et al.*, 1985).

It has been suggested that there are two linear phases of denitrification. Phase I is the initial linear phase, occurring once O₂ inhibition of *in situ* denitrifying enzymes is removed, lasting from approximately 15 minutes to 1 to 8 hours (Smith and Tiedje, 1979; Limmer and Steele, 1982). During Phase I, N₂ has been observed to be the dominant gaseous end product of denitrification (Firestone and Tiedje, 1979). Phase I is a measure of the activity of existing enzymes, and the increase in rate following Phase I is considered to be due to the synthesis of denitrifying enzymes (Firestone and Tiedje, 1979; Smith and Tiedje, 1979).

Phase II, reflecting denitrification potential, is thought to commence after 4 to 8 hours, once each cell has reached its maximum capacity to denitrify (Smith and Tiedje, 1979; Limmer and Steele, 1982). An increase in denitrification rate during Phase II requires an increase in denitrifying population, and therefore a supply of available C (Smith and Tiedje, 1979).

2.2.3 Factors affecting denitrification

Denitrification can be affected by a number of factors including oxygen availability and substrate (carbon and NO₃⁻) availability. These factors can then be affected by a wide range of additional soil characteristics, many of which can be significantly affected by agricultural activities.

2.2.3.1. Soil moisture

Soil moisture is an important factor in the regulation of biological denitrification (Rolston, 1981). Soil moisture content influences denitrification directly by providing an environment suitable for micro-organisms through its effect on nutrient availability, O₂ tension, and microbial movement (Bremner and Shaw, 1958a; Rolston, 1981).

Bacteria are only capable of moving rapidly over surfaces when the thickness of the water film is comparable to that of the bacterial cell (Lynch, 1988). As the soil water content increases, there is a corresponding rise in substrate solubilisation and nutrient movement (Tiedje *et al.*, 1984), coupled with a decrease in gaseous diffusion as pores become waterlogged. These conditions can lead to the formation

of anoxic microsites, which favour denitrifier activity, as a result of microbial respiration of O₂ exceeding supply by diffusion (Rolston, 1981; Lynch, 1988).

Rainfall and irrigation usually cause an increase in the denitrification rate as the soil water content peaks, provided other factors, e.g. N oxides and degradable organic material, are not limiting (Freney *et al.*, 1979; Rolston *et al.*, 1982; Mosier *et al.*, 1986). The effect of soil moisture content on denitrification rate has been widely investigated. Some authors have found no or only a weak, correlation between soil water content and rate of denitrification (Limmer and Steele, 1982; Hixson *et al.*, 1990). However, in most studies a strong and positive correlation was reported (Bremner and Shaw, 1958a; Myrold, 1988; Davidson, 1993).

Denitrification rates have been found to increase in soils as the moisture content exceeds 25 (Smith and Tiedje, 1979) to 60 % of water holding capacity (Bremner and Shaw, 1958a; Freney *et al.*, 1979), reaching a plateau once soil becomes waterlogged (Payne, 1981a). Nõmmik and Larsson (1989) reported that denitrification in intact cores of forest topsoil decreased when the moisture content was lowered below 100 % water holding capacity, and an increase above 100 % led to a 1 to 28 times increase in denitrification.

Studies at the University of Edinburgh and elsewhere have looked at the effect of moisture on N₂O and N₂ emissions from different soils. It was concluded that between 60 and 80 % water filled porosity there was a very significant increase in N₂O concentrations, whereas an increase from 80 to 90 % water filled porosity led to some further increase in N₂O, but also a substantial increase in N₂ production because of the further reduction of N₂O (Colbourn, 1992; Dobbie *et al.*, 1999; Abbasi and Adams, 2000a; Weitz *et al.*, 2001).

Denitrification rates from the top 0-5 cm soil depth measured under forest, fallow and agricultural systems with very similar climate and parent soil characteristics in central Germany, by Teep *et al.* (2000), were elevated during periods of both soil freezing and soil thawing. The N₂O emission rates during the entire winter period were 16, 25 and 60 µg N₂O-N m⁻² h⁻¹ for forest, fallow and agricultural land,

respectively. In addition, the average water-filled pore space (WFPS) in the top 0-5 cm of soils was found to be positively correlated with the total N₂O winter emissions. This indicates the importance of oxygen diffusion as a regulator for N₂O winter emissions. The difference in N₂O winter emissions among soil systems might be due to the differences in water-filled pore space (WFPS) under these systems. Another possibility might be due to delayed soil freezing of about 3 to 4 weeks because of the upper organic layers of the forest and the grass vegetation of the fallow system, and deeper frost penetration compared to the agricultural land.

2.2.3.2 Microbial population

The most common method of counting denitrifiers is the most probable number (MPN) technique (Focht, 1982). The population size of NO₃⁻ reducing bacteria, as determined by MPN, has been observed to have no correlation with potential NO₃⁻ reduction activity in soil bacteria (Terry and Tate, 1980). However, Yeomans *et al.* (1992) showed there was a positive correlation ($r = 0.70$) between denitrifier populations, as enumerated by MPN, and the ability of deep profile (0-300 cm) soil samples to denitrify NO₃⁻ over 72 hours.

Enumeration of denitrifiers is perhaps of little use as population density may have no direct relationship with denitrifying enzyme concentration or activity (Tiedje, 1982). As denitrifiers are largely aerobes, their enumeration from soil samples has no direct quantitative relationship with denitrification (Focht, 1982), which depends largely on environmental conditions around the denitrifying microsite.

Kromka *et al.* (1992) looked at the reduction rate of nitrous oxide in different soil groups during denitrification and found that most of the biomass of denitrifiers (80-90 %) in different soil groups was able to reduce nitrous oxide. An exception was salt-affected soil (Solonchak) where the biomass of denitrifiers reducing nitrous oxide was only half (54-56 %). This suggests that denitrifiers in salt affected soils have some peculiarities and denitrification in salt affected soils proceeds mainly to nitrous oxide.

2.2.3.3 Soil type and structure

Soil texture influences the denitrification process in two ways; Firstly, exposed soil surfaces provide attachment sites for microbial cells. Secondly, the negatively charged soil colloids may concentrate nutrients in the soil solution (Focht and Verstraete, 1977). Greater rates of denitrification have been reported in finer-textured soils (Lund *et al.*, 1974). These greater rates may not be a direct effect of the clay on micro-organisms, since Van der Staay and Focht (1977) were unable to show any differences in denitrification rates between clays of different particle sizes after removal of indigenous organic matter and subsequent addition of substrate and inoculum, while the higher organic matter content of these finer textured soils coupled with generally slower drainage may lead to restricted aeration in micropores and therefore the likelihood of more anoxic conditions and increased denitrification. O₂ diffusion into aggregates or other denitrifying sites may be a major factor controlling denitrification in soils (Parkin and Tiedje, 1984).

The soil system can be represented as groups of spherical soil aggregates, with oxic outer shells surrounding an inner core that may be anoxic (Greenwood, 1978). The degree of anoxia within these aggregates is thought to expand and contract in response to O₂ supply and/or consumption (Tiedje *et al.*, 1984). Denitrification within these aggregates varies with the relative proportions of oxic and anoxic volumes, and the rate of metabolic activity and diffusion of NO₃⁻ into the anoxic regions. Burton and Beauchamp (1985) found that an air-filled porosity in excess of 35 % allowed sufficient supply of O₂ to inhibit denitrification in cores of silty loam topsoil, while Prade and Trolldenier (1988) observed low denitrification rates below 10 to 12 % air-filled porosity in loam soils planted with wheat. Below 10 to 12 % air-filled porosity, denitrification was seen to increase exponentially as air-filled porosity decreased (Prade and Trolldenier, 1988).

Soil type has been reported to have a significant effect on N₂O concentrations, with larger N₂O fluxes reported from lighter textured soils compared to heavier soil (Nõmmik and Larsson, 1989; Arah *et al.*, 1991). N₂O fluxes from coarser soils may appear higher due to faster diffusion of N₂O out the soil profile and the possibility of further reduction of N₂O to N₂ during the slow diffusion out of heavier soils.

2.2.3.4 pH

The optimum pH for denitrification varies with species of organism and NO_3^- concentration (Delwiche and Bryan, 1976). The range pH 6 – 8 is generally suggested to be optimal for denitrification in soil (Bremner and Shaw, 1958a; Nägele and Conrad, 1990), with pure cultures of soil denitrifiers exhibiting an optimum pH of around neutrality (Nägele and Conrad, 1990).

Below a pH of 5, denitrification appears to be considerably reduced in soils (Bremner and Shaw, 1958) and has been observed to occur very slowly in acid soils and peats with pHs of 3.6 - 4.8 (Bremner and Shaw, 1958a, Klemetsson *et al.*, 1977). Parkin *et al.* (1985) observed that while denitrification rates in sandy loam soil with pH 3.9 were greatly reduced compared to rates from the same soil at pH 6, they could still account for significant losses of $158 \pm 216 \text{ mg N kg}^{-1} \text{ day}^{-1}$. While NO_3^- reduction potential decreases with pH (Nägele and Conrad, 1990), NO_3^- reducing bacteria remain active in acid soils, with the bacteria exhibiting a broad range of pH tolerance.

Denitrifying activity observed at low pH has been suggested to be due to either denitrifier populations whose activity is reduced by the pH, small populations of denitrifiers protected within neutral pH microsites, or denitrifiers with low pH optima (Nägele and Conrad, 1990). Nägele and Conrad (1990) suggested that the increased activity of NO_3^- reducers following an increase in pH in acid soil indicated that the NO_3^- reducing bacteria were not specifically adapted to low *in situ* pH, and that the increase in NO_3^- reduction was due to a rise in activity, as opposed to an increase in microbial population size. However, Parkin *et al.* (1985) observed that in soil with long-term (20 years) exposure to pH <4, denitrifier populations appeared to have adapted to acid conditions, as denitrifying activity was measured in slurries of non-aggregated sandy loam soil where neutral pH microsites were unlikely to exist. Parkin *et al.* (1985) suggested that different denitrifier populations existed in low pH soils, and observed that denitrification activity was not limited by pH in such soils, and indeed was optimal at the pH closest to natural levels.

2.2.3.5 Influence of agricultural practices

Both cultivation procedures and the species of crop grown can affect the rate of denitrification. It has been suggested that the response of field denitrification to different treatments is due to the effect of various practices on soil aeration, C availability and NO_3^- supply (Myrold, 1988; Granli and Bøckman, 1994). Cultivation may result in an increase in the denitrification rate as it enhances the availability of incorporated residues to soil micro-organisms, which may result in the low O_2 tensions conducive to denitrifier activity within microsites.

Surface tillage practices have been observed to have no significant influence on microbial activity in the subsoil (Parkin and Meisinger, 1989). However, ploughing and cultivation of the topsoil results in increased aeration and evaporation of moisture (Granli and Bøckman, 1994), and therefore reduces the likelihood of denitrification. Palma *et al.* (1997) observed that denitrification losses under a no-tillage (NT) system were twice as great as those from a conventional tillage (CT) system. Similarly Arah *et al.* (1991) found that N_2O concentrations from depth probes at 5, 20 and 40 cm depth were generally greater in direct-drilled compared to ploughed plots. This may be due to the higher water content and lower total porosity generally observed in direct-drilled and undisturbed soils compared to ploughed soils (Granli and Bøckman, 1994).

Denitrification potential in cropped soils has been reported to be three to seven times greater than that in fallow fields (Terry and Tate, 1980), with the increase in denitrification thought to be due to the litter of decaying plant residues and dying roots acting as an additional source of C. Myrold (1988) observed that denitrification in a soil under winter wheat accounted for three times as much of the applied fertilizer N as denitrification under ryegrass. It has been suggested that these differences in denitrification are due to the different growth and development patterns of various crops (Mosier *et al.*, 1986; Myrold, 1988).

Bakken (1988) observed that at low water holding capacity plant roots had a slightly negative effect on denitrification, at moderate water capacity there was no stimulatory effect, while at 100 % water holding capacity the presence of roots

resulted in a ten-fold increase in denitrification. Negative effects of plant roots may be due to the removal of NH_4^+ and NO_3^- from soil (Smith and Tiedje, 1979), and also due to water consumption, resulting in reduced soil moisture (Firestone and Davidson, 1989), and the improvement of soil aeration through the formation of root channels (Granli and Bøckman, 1994). Therefore, if NO_3^- is the factor limiting denitrification in a soil, plants may out-compete denitrifiers for N, but where the system is C-limited, root exudates may stimulate denitrification (Firestone and Davidson, 1989).

2.2.3.6. Irrigation water quality

Irrigation with low water quality such as wastewater has produced both desirable and undesirable effects on the environment. The fate of nitrogen applied to soils through wastewater irrigation is an important issue in wastewater reuse and groundwater contamination. Bole *et al.* (1981) found that a gradual build-up of soil NO_3^- -N under wastewater irrigation leads to an increase in its concentration in surface and groundwater. Thus, denitrification is a desirable process under wastewater irrigation to reduce NO_3^- -N. The final N loss through the denitrification process from effluent irrigated fields depends greatly on soil and environmental conditions.

Feigin and Kipnis (1980) observed that loss of nitrogen from a Rhodes grass field through denitrification was much higher (up to 50 % of the nitrogen applied in effluent and fertilizer) than in a cotton field. There are many possible reasons for this; Firstly, the quantity of effluent and consequently the organic carbon applied to the grass was twice that given to the cotton. Secondly, the irrigation frequency was much greater in grass (intervals of 3-7 days in grass and 2-3 weeks in cotton) and the grass field topsoil retained water for longer periods. Thirdly, the grass was cut every 21 days during the active growing season, thus increasing available carbon in the soil through plant residues and dead roots. Finally, the oxygen level in the 0-0.9 m soil layer was much lower in the Rhodes grass field than in the cotton field, where it was greater than 14 %.

Feigin *et al.* (1981) observed an increase in nitrogen loss from the applied nitrogen fertilizer to a Vertisol (70 % clay, 0.11 % total N, 1.6 % organic matter), in a greenhouse experiment after irrigation with sewage effluent (total N 70 mg l⁻¹, NH₄⁺-N 49 mg l⁻¹, organic N 21 mg l⁻¹, total organic carbon 93 mg l⁻¹), compared to N loss under irrigation with mineral solution simulating the mineral composition of the sewage effluent (total N 54 mg l⁻¹, NH₄⁺-N 54 mg l⁻¹, organic N 0 mg l⁻¹, organic carbon 0 mg l⁻¹). This might be due to the combined effect of a build-up in NO₃⁻-N, and an increase in anaerobic conditions in the soil under sewage effluent irrigation because of the application of carbon and high biological oxygen demand of sewage stimulating denitrification and increasing loss of nitrogen.

2.2.3.7 Nitrogen source

Nitrate is the preferred electron acceptor for denitrifiers (Bailey and Beauchamp, 1973). Bremner and Shaw (1958a) showed that the source of NO₃⁻ in soil is not important, with potassium, sodium and calcium nitrates giving almost identical results. Additions of urea and NH₄NO₃ have also been observed to result in an increase in denitrification (Lindau *et al.*, 1988; Colbourn, 1992). Denitrification rate has been increased by the application of a liquid rather than a dry granular form of N-source (Paramasivam *et al.*, 1998). This is due to the readily available nature of the N in the liquid form. Inhibitory effects on NO₃⁻ reduction have been observed in the presence of NO₂⁻ (Bailey and Beauchamp, 1973) and NO (Nõmmik *et al.*, 1984).

The nitrogen source has been reported to have little qualitative effect on nitrogenous gas production (Bailey and Beauchamp, 1973). Nitrate is reduced preferentially, and is accompanied by a transient accumulation of NO₂⁻ (Payne, 1981a). Addition of NO₃⁻ to soils with no background NO₃⁻ content has been reported to result in the stoichiometric production of N₂O, which increases in proportion to the concentration of NO₃⁻ (Müller *et al.*, 1980). Where organic matter is in short supply, the electron donor source becomes the limiting factor and there is no effect of increasing NO₃⁻ concentration on denitrification rate (Limmer and Steele, 1982; McCarty and Bremner, 1992).

2.2.3.8 Organic matter

Heterotrophic growth in soils is generally considered to be limited by the availability of a carbon (C) source (Lynch, 1988). As the majority of denitrifiers are heterotrophs, an organic C source (to act as an electron donor) may be more important than O₂ to the density of the heterotrophic denitrifier population (Tiedje *et al.*, 1982). Supply of organic matter can influence denitrification either directly as a growth substrate, or through increased microbial activity and O₂ consumption, resulting in the formation of anoxic microsites (Rolston, 1981).

Denitrification has been observed to be significantly correlated with total organic C, water-soluble organic carbon (WSOC) and mineralisable C (Burford and Bremner, 1975; Beauchamp *et al.*, 1980). Concentrations of WSOC in excess of 40 to 80 µg C g⁻¹ dry soil have been reported to be necessary before denitrification is observed in soils (Beauchamp *et al.*, 1980; Burton and Beauchamp, 1985).

Under aerobic conditions, a wide variety of organic acids, carbohydrates and other organic compounds can be utilised by denitrifying bacteria. However, under anaerobic conditions these organisms may be restricted to fewer C sources (Beauchamp *et al.*, 1989), and denitrification rates will depend largely on the amount of readily available organic soil C (Bremner and Shaw, 1958a; Paul *et al.*, 1989). In a study with 32 field-moist and air-dried soils from a range of arable and grasslands, Bijay-Singh *et al.* (1988) reported that while air-dried soils showed the greatest correlations between denitrification potential and water-soluble C and aerobically mineralized C, the field-moist soils showed the closest correlation of denitrification potential with C mineralized under anaerobic conditions. The authors suggested that, in the field, denitrification may often be limited by the amount of C susceptible to mineralization under anaerobic conditions.

The decomposition rate of different forms of organic matter by micro-organisms varies greatly, with readily available plant exudates taking minutes to hours, while crop residues and manure taking days, months, or years, and humus needing centuries (Granli and Bøckman, 1994). Readily decomposable and water-soluble constituents of organic matter are considered to be the most effective in promoting

denitrification (Bremner and Shaw, 1958a). Different organic residues with similar concentrations of extractable C have been observed to result in similar rates of N₂O emissions (deCatanzaro *et al.*, 1987).

Bacterial cells require and assimilate C and N in a ratio of approximately 1:5 (Wood, 1989). Therefore, N-containing organic matter tends to be more rapidly decomposed than non-nitrogenous compounds (Gray and Williams, 1971). The C: N ratio in soil is approximately 10:1 (Schepers and Mosier, 1991). In low C: N ratio systems, the reductant-limiting conditions favour denitrification, while high C: N ratios in excess of 20 favour immobilization (Lynch, 1988). Organic matter concentration tends to be greatest at the soil surface, therefore resulting in a decrease in denitrification potential down the soil profile (Rolston, 1981).

Animal wastes may promote denitrification by creating anaerobic environments and a source of C (Campbell, 1983; Beauchamp *et al.*, 1989). Manure largely consists of the undigested remains of animal feed and bedding, as well as high microbial populations, which would not be expected to act as readily available C sources for denitrifiers (Beauchamp *et al.*, 1989). However, following addition of manure to soil, microbial death due to environmental change may result in increased availability of microbial C to denitrifiers. Major plant components such as cellulose and hemicellulose may also require the action of other micro-organisms, such as cellulose fermenters, before the products of the cellulases and fermentation processes are readily available to denitrifying micro-organisms (Beauchamp *et al.*, 1989).

2.2.3.9 Temperature

As with most biological processes, the denitrification rate increases with increasing temperature until an optimum is reached above which it decreases. Temperature in soil is determined by ambient air temperature and depth. Temperature affects the physiological reaction rates of cells and the physico-chemical characteristics of the environment; soil volume, pressure, oxidation-reduction potentials and diffusion (Paul and Clark, 1989). High temperature leads to a reduction in soluble O₂ and N₂O concentration as well as an increase in biological rate processes (Focht, 1982).

The lower solubility of N_2O within soil water at higher temperatures would result in more N_2O diffusing out of the soil profile into the atmosphere, which may partly explain the reported increase in denitrification at higher temperatures.

Denitrification rates increase rapidly between 2 and 37 °C, reaching an optimum at 25 to 30 °C (Bremner and Shaw, 1958a; Freney *et al.*, 1979). Bailey and Beauchamp (1973) observed a rapid decrease of denitrification rate below 5 °C. In field studies with different tillage and cropping systems, Aulakh *et al.* (1983) and Aulakh and Rennie (1984) observed little influence of temperature on denitrification rate during the crop-growing season when temperatures ranged from 10 to 30 °C. However, in early spring and late fall when temperatures were 5 °C or below, virtually no denitrification was detectable, even in wet soils with high NO_3^- content.

Increasing temperature has been observed to result in a decrease in NO_2^- accumulation (Dorland and Beauchamp, 1991), and Melin and Nõmmik (1983) reported that N_2O was the dominant gas from incubations at 4 °C, while at 20 °C equal concentrations of N_2O and N_2 were observed.

Reduction of N_2O has been reported to have a Q_{10} of 2.1 (i.e. the ratio of the rate of N_2O reduction at one temperature to that at a temperature 10 °C lower) between 15 and 20 °C in clay and sandy loam soils (McKenney *et al.*, 1984). Further increases in temperature above 30 °C have little effect, until lethal temperatures above 60 to 70 °C are reached (Bremner and Shaw, 1958a). In glucose-amended soils of varying organic matter and physical and chemical characteristics, Stanford *et al.* (1975) found that the denitrification rate was minimal at 0 to 5 °C, but increased ten-fold between 5 and 10 °C. Between 15 and 35 °C the temperature coefficient of denitrification (Q_{10}) was approximately 2, and between 35 and 45 °C, denitrification rates varied very little. Focht and Chang (1975) concluded that the Q_{10} for denitrification in soil was in the range of 5 - 16 for temperatures below 12-15 °C. However, others found that the denitrification rate increased with rising temperature to a maximum between 65 and 75 °C and decreased rapidly to zero with further temperature increases above this maximum. (Bremner and Shaw, 1958a; Keeney *et al.*, 1979).

Substantial denitrification rates have been reported at -2 °C in unfrozen, supercooled samples, especially in those amended with a carbon source (Dorland and Beauchamp, 1991). It has been suggested that as organic matter content decreases, the threshold temperature for denitrification increases (Dorland and Beauchamp, 1991). An alternative theory for the different reported threshold temperatures is the adaptability to low temperatures of denitrifiers from different climatic regions (Dorland and Beauchamp, 1991).

2.2.3.10 Oxygen

Soil is a heterogenous habitat and usually contains both aerobic and anaerobic sites. Soil aeration status depends upon factors such as soil moisture content, diffusion of O₂ into the soil, and consumption of O₂ by soil micro-organisms and plant roots (Smith, 1990). Aeration is the greatest and most variable factor that affects the denitrification process in soil (Focht, 1982). Oxygen is thermodynamically preferred to NO₃⁻ as an electron acceptor due to its higher energy potential (Focht, 1982). As conditions tend towards anoxia, facultative anaerobes decompose organic matter, using oxidised soil components as electron acceptors in respiration (Stolzy and Fluhler, 1978).

The presence of available organic matter may also decrease soil O₂ if it results in an increase in aerobic microbial respiration where O₂ consumption exceeds supply (Parkin, 1987). In waterlogged soils, O₂ demand can exhaust dissolved O₂ within 24 hours (Bohn *et al.*, 1985). However, overall aeration of a soil is less important than that of individual crumbs and aggregates.

Denitrification requires anaerobic conditions and occurs when the supply of oxygen required by the soil micro-organisms is restricted (Bremner and Shaw, 1958a). Some of the most common denitrifiers in soil are *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Thiosphaera pantotropha*, *Pseudomonas denitrificans*, *Alcaligenes faecalis* and *Pseudomonas aureofaciens* (Robertson *et al.*, 1989). Denitrifiers have different sensitivities to dissolved O₂ concentrations. Many micro-organisms require very low O₂ concentrations or even anoxia (Kuenen and Robertson, 1988), while

others like *Thiosphaera pantotropha* are capable of co-respiring O_2 and NO_3^- or NO_2^- (Robertson and Kuenen, 1990).

It is thought that as O_2 becomes limiting, denitrifiers possess the ability to simultaneously synthesise denitrifying enzymes (Payne, 1973). The presence of O_2 reversibly represses denitrifying enzyme synthesis and activity, restricting NO_3^- and NO_2^- reduction (Hixson *et al.*, 1990). The specific regulatory control of the N oxide reductases is not clearly understood, but it appears that there are always small quantities of these enzymes present (Focht, 1982) and that the critical O_2 tension before enzyme synthesis depends on the denitrifying organism and the enzyme system involved (Robertson *et al.*, 1989).

Several studies investigated the effect of oxygen on denitrification (Betlach and Tiedje, 1981; Parkin and Tiedje, 1984; Burton and Beauchamp, 1985; Arah *et al.*, 1991). Parkin and Tiedje (1984) showed that denitrification rates increased 1 to 5 times when the O_2 concentrations in cores decreased from 20 to 5 %. They suggested that O_2 diffusion into aggregates and sites of denitrification may be a major factor controlling denitrification in soil. Denitrification rates in soil cores approached anaerobic rates when the O_2 concentration fell below 0.5 % (Parkin and Tiedje, 1984). In pure denitrifier cultures lag phases of between 95 and 205 minutes have been observed before N_2O production occurs, following a shift from oxic to anoxic conditions (Smith and Tiedje, 1979). It has been shown that phase I of denitrification responds to altered soil aeration, whereas phase II is unaffected (Smith and Tiedje, 1979).

Firestone and Tiedje (1979) suggested that there was a staggered approach to synthesis of denitrifying enzymes in response to anoxia, with the switch to NO_3^- reductase preceding that to N_2O reductase. However, pure culture incubations with *Pseudomonas fluorescens* indicated that the sensitivity of denitrifying enzymes to O_2 concentration increased in the same order as the denitrification pathway: NO_3^- reductase was less sensitive than NO_2^- reductase, which was less sensitive than NO reductase, with N_2O reductase being the most sensitive enzyme to O_2 (McKenney *et al.*, 1994). The increased inhibition of N_2O reductase in the presence of O_2 results in

an increase in the $\text{N}_2\text{O}:\text{N}_2$ ratio with increasing O_2 concentration (Betlach and Tiedje, 1981; Granli and Bøckman, 1994).

2.2.3.11 Water table fluctuation

The denitrification process is dependent on the oxygen availability in the soil. Excess water in soil excludes air, resulting in anaerobic conditions. Therefore, the position of the water table (the level below the land surface at which the subsurface material is fully saturated with water) is an important factor controlling denitrification rate (Martikainen *et al.*, 1995).

In arid and semi-arid regions, the main reason for a rise in the water table is due to the misuse of irrigation water without adequate drainage (Elgabaly, 1970). In the absence of irrigation, the denitrification process in these regions is possibly decreased because of frequent summer drought or increased evaporation and evapotranspiration, which will therefore result in a lowering of the water table, and cause an increase in the oxygen availability in the upper soil layers. N_2O is produced under partially anaerobic conditions ($0.250 \text{ V } E_h$) (Mitsch and Gosselink, 1993), but relatively few studies have investigated the potential effect of lowering the water table on denitrification rate (Martikainen *et al.*, 1995; Aerts and Ludwig, 1997). Aerts and Ludwig (1997) found that N_2O -emission from columns of peatland soils was increased significantly ($p < 0.05$) by the relatively small lowering of the water table to 10 cm below the surface. However, at a high static water table (at the peat surface) it was not significantly different. This might be due to the further reduction of N_2O to N_2 because of a decrease in oxygen availability.

In another study, Elmi *et al.* (2000) observed that the shallow water tables enhanced the denitrification process. As a result, NO_3^- concentrations were reduced by 16 – 42 % over a 2 year period compared to treatments involving free drainage.

2.2.4 Denitrification products

Nitrogen is lost from soils as either nitric oxide (NO), nitrous oxide (N_2O) and/or nitrogen gas (N_2). The denitrification products NO_2^- and NO are generally considered to be transient products. Under anoxic conditions NO_2^- is further

reduced to NH_3 or N_2O . However, if carbon is limiting, NO_2^- tends to accumulate in soil (Stolzy and Fluhler, 1978). NO is highly soluble, reactive and rapidly decomposed by O_2 (Payne, 1981a) and is therefore rarely observed as a denitrification product. In contrast, N_2O and N_2 are the dominant products of denitrification and the preferred end product is N_2 . This is probably due to the greater energy yielded by its synthesis (Delwiche, 1981).

The final products of denitrification can be affected by the environmental conditions surrounding the denitrifying microsite. N_2O production is favoured over N_2 at low temperature, low pH and in the presence of O_2 (Betlach and Tiedje, 1981; Granli and Bøckman, 1994), conditions that are marginal for denitrification, and may therefore not result in significant fluxes of N_2O to the atmosphere, even though it is the dominant end product.

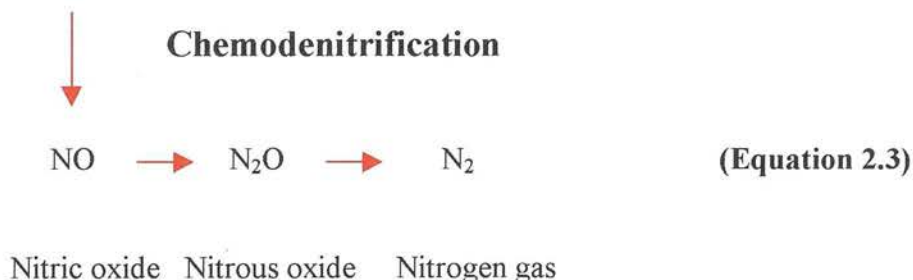
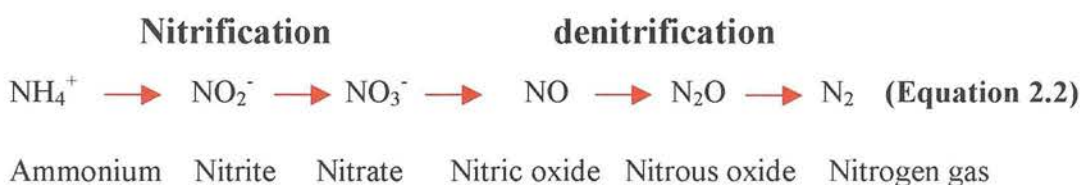
Under acidic conditions, NH_4^+ is a major product of NO_3^- reduction (Bremner and Shaw, 1958a), with only 3 to 10 % of NO_3^- added to soil of pH 4.5 or less reduced to N_2O (Müller *et al.*, 1980). Production of NO can be enhanced in low pH soils (Delwiche, 1981). As pH increases above 6.6, the amount of N_2 relative to N_2O increases (Burford and Bremner, 1975; Rolston, 1981).

At low C: N ratios, the release of N_2O is favoured (Delwiche, 1981), while high C: N ratios favour N_2 (Rolston, 1981). Low NO_3^- concentrations favour N_2 (Rolston, 1981) as N_2O may be used as the terminal electron acceptor in the absence of NO_3^- , with the N_2O : N_2 ratio increasing with the level of NO_3^- . Concentrations of $\text{NO}_3^- > 50 \text{ mg kg}^{-1}$ have been reported to have an inhibitory effect on N_2O reduction (Blackmer and Bremner, 1979). It is not known if this effect is due to high concentrations of NO_3^- inhibiting N_2O reductase, or because denitrifiers energetically prefer to reduce NO_3^- to N_2O (Granli and Bøckman, 1994). Betlach and Tiedje (1981) proposed that the accumulation of NO_2^- and N_2O in pure denitrifier cultures could be determined by reaction rates rather than inhibition of steps in the denitrification sequence. They suggested that NO_2^- accumulation was due to the slower reduction rate of NO_2^- compared to NO_3^- , and not NO_3^- inhibition of NO_2^- reduction.

2.2.4.1 Processes involved in the formation of N₂O

Production of N₂O is related to N transformations occurring in the soil, with high N₂O emissions being associated with high transformation rates of N (Riley and Vitousek, 1995). Denitrification and nitrification are the sources of N₂O, and these processes can occur simultaneously in soils (Abbasi and Adams, 2000a, b). Until 1980, denitrification was regarded as the main source of atmospheric nitrous oxide (N₂O) but work reported by Bremner and Blackmer (1981) showed that nitrification can also be a significant source of N₂O.

Nitrification occurs in well-aerated soils and involves the oxidation of NH₄⁺ to NO₂⁻ and NO₃⁻ (Bouwman, 1990a), as shown by the reactions below.



Under aerobic conditions, nitrification can be the dominant N₂O producing process (Bremner and Blackmer, 1978; Mosier *et al.*, 1998). Denitrification, on the other hand, occurs where there is no oxygen or where O₂ is partially limited. Nitrification and denitrification processes can occur simultaneously at different rates, depending on the soil moisture content (Abbasi and Adams, 2000a, b). Generally, denitrification contributes more N₂O than nitrification where O₂ is limited (Bauhus *et al.*, 1996).

2.3 Methodology

2.3.1 Measurement of denitrification in the laboratory

Denitrification is a difficult process to measure due to the high ambient atmospheric concentration of N_2 , a major product of denitrification. Therefore, alternative methods are used in laboratory studies based on soil core incubations in closed systems, where the gaseous products of denitrification evolve into a confined atmosphere. These losses are determined either by direct mass spectrometry of $N_2 + N_2O$ (Arah *et al.*, 1993), by the acetylene inhibition technique (Yoshinari *et al.*, 1977) and gas losses measured by GC of N_2O , or by using a N_2 -free atmosphere technique in which gases of the helium group are commonly used to establish such an atmosphere (Scholefield *et al.*, 1997).

Direct mass spectrometry is based on the addition of ^{15}N -enriched NO_3 to the soil and measurements of ^{15}N -enriched N_2O and N_2 fluxes using a mass spectrometer. The acetylene inhibition technique is based on the inhibition of the N_2O reductase enzyme and measurement of N_2O fluxes using gas chromatography; this technique is very sensitive, thus concentrations as low as the atmospheric concentration of N_2O (0.31 ppm) can be detected. The last procedure is based on replacement of N_2 in the pores of intact soil cores by continuous flushing of the headspace gas of the incubation system with a stream of helium group gases. A mixture of 20 % O_2 in helium has been used in this technique, and N_2O fluxes have been measured using a gas chromatograph (GC) fitted with an electron capture detector (ECD), while N_2 was determined using another GC fitted with a He-purged injection valve and katharometer detector (Scholefield *et al.*, 1997).

2.3.1.1. Acetylene inhibition method

The use of C_2H_2 to inhibit the reduction of N_2O to N_2 has been used to measure total denitrification in laboratory incubations (Klemetsson *et al.*, 1977). This was found to be a very useful technique to avoid the problems associated with sample contamination by high ambient atmospheric N_2 (Klemetsson *et al.*, 1977; Yoshinari *et al.*, 1977). This technique is less expensive than the ^{15}N -tracer technique. In contrast, acetylene inhibition is not recommended for long-term studies. This is due

to its transient nature and adverse effects on soil microbial metabolism (Focht, 1982). Although denitrifiers can not use C_2H_2 itself as a source of carbon (Yeomans and Beauchamp, 1982), under oxic conditions other micro-organisms may metabolise C_2H_2 to ethanol, acetate or acetaldehyde, which can be used as C sources by denitrifiers (Topp and Germon, 1986).

Klemedtsson *et al.* (1990) described the 'ppm method', the use of different concentrations of C_2H_2 to quantify N_2O production by nitrification, and N_2O and N_2 production by denitrification. Concentrations from 0.001 to 0.1 % of acetylene totally inhibits nitrification in soil (Walter *et al.*, 1979; Klemedtsson *et al.*, 1988). Reduction of N_2O is inhibited by 1 to 10 % C_2H_2 (Klemedtsson *et al.*, 1977; Yoshinari *et al.*, 1977), allowing the measurement of total denitrification.

The exact mechanism by which acetylene inhibits N_2O reductase is not clear. Focht (1982) reported that the inhibitory effect is reversible, and it is thought that the resumption of N_2O reduction may be attributed to denitrifiers adapting and becoming insensitive to C_2H_2 or the presence of a large biomass or enzyme concentration that is no longer saturated by C_2H_2 .

2.3.1.2. Isotopic methodology

The heavy stable isotope of N (^{15}N), with a natural abundance of 0.3663 %, is the most commonly used tracer in soil investigations as the half-life of radioactive N isotopes is too short, e.g. ^{13}N has a half-life of 10 minutes (Jansson, 1971).

The use of ^{15}N as a tracer was found to be the best technique to differentiate between the fate and cycling of added ^{15}N compared to native N, through mass spectrometric analysis (Myrold, 1990). ^{15}N tracer was also found to be useful in distinguishing between nitrification and denitrification sources of N_2O using measurements of $^{15}N_2O$ as well as soil ^{15}N pools and N_2 emissions during denitrification (Panek *et al.*, 2000). However, the main disadvantage of stable isotope methodology is the expense of highly enriched ^{15}N and also the fact that any addition of ^{15}N to the soil-N pool can alter the nitrogen concentration of the soil system under study unless labelled N is added as an alternative to unlabelled fertilizer. Another problem

associated with ^{15}N methodology is the tendency of ^{15}N molecules in biological systems to react more slowly than ^{14}N molecules, therefore substrates may become enriched with ^{15}N (Killham, 1994). Denitrifiers in culture and soil have been observed to preferentially reduce ^{14}N at a fractionation rate approximately between 1.02 and 1.005 (Payne, 1981a). This discrimination is inconsequential where there is an excess supply of ^{15}N , but may be of importance where there is low ^{15}N enrichment (Payne, 1981a).

2.4 Aims of the work presented in this thesis

Many gaps exist in our knowledge of microbial reduction of nitrate in surface irrigated soils. Thus, this study aimed to increase understanding of the microbial reduction of nitrate (denitrification) in topsoil irrigated with different water qualities (wastewater, fresh water and river water), and the factors that control this process at two sites in Greece (the Galicos River Wastewater Treatment Project and Axios Delta fields). This study takes into account the effects of oxidizable organic carbon, soil moisture, soil structure and soil texture variability. Such studies could provide data that shows the effect of current agricultural management on soil properties. This data would be useful for future agricultural practice, focused at limiting nitrogen losses from irrigated soils.

Because wastewater has medium to high salinity accompanied by high concentrations of sodium, municipal wastewater irrigation may cause increased soil salinity, sodicity, alkalinity and specific ion toxicity. Wastewater also contains soluble and insoluble components, such as salts, nutrients, trace elements and organic compounds that may alter the irrigated soil properties. The first aim was to test the hypothesis of changes in physical and chemical surface soil (0-5 cm depth) properties occurring over a four year period of wastewater irrigation at the Galicos River site compared to the properties of soil irrigated with well water.

The second aim was to test the hypothesis that the use of wastewater is expected to increase denitrification rates because of its high biological oxygen demand (BOD_5) and high sodium content which has the ability to disperse soil, resulting in reduced hydraulic conductivity of the soil and water infiltration rate, and also leads to poor

aeration. All together, these would enhance denitrification rates. To test this second hypothesis, loose soil samples were collected from plots irrigated for four years either with wastewater or well water from the Galicos river site and were repacked in metal rings. Denitrification rates were measured from these repacked soil cores after N-amendment to find out if the irrigation with wastewater would result in increased denitrification rates compared to well water irrigation

A third hypothesis is that the supply of organic matter can increase denitrification rates either directly as a growth substrate for denitrifiers, or through increased activity and O₂ consumption, resulting in the formation of anoxic microsites. To test this hypothesis, amendments of organic carbon were applied to intact topsoil cores from plots irrigated over four years either with wastewater or well water from the Galicos river site to examine if addition of available organic carbon to intact topsoil cores would result in increased topsoil denitrification compared to those in the presence of natural carbon concentrations.

Greater denitrification rates would also be expected to occur in fine textured soils, due to the increased possibility of the occurrence of anaerobic microsites in these soils. To test this fourth hypothesis, clay, clay loam and sandy loam intact topsoil cores were collected from fields in the Axios Delta site, irrigated naturally by the flooding of the Axios River. Denitrification rates in these differently textured intact soil cores were monitored in the presence of natural organic carbon concentrations.

The proposed aims necessitated measurements of total denitrification. Because of a lack of adequate equipment such as a mass spectrometer (MS) at the Scottish Agricultural College (SAC) Edinburgh, it was decided to use the C₂H₂ inhibition method for measuring total denitrification in laboratory incubations (Klemmedtsson *et al.*, 1977; Yoshinari *et al.*, 1977).

3 GENERAL MATERIALS AND METHODS

3.1 Soil origin

Loose soil samples and intact cores were collected by staff from the Galicos River Wastewater Treatment Project, located at 40° 40' N latitude, 22° 48' E longitude in the Thessaloniki plain in northern Greece. The soils were taken from two sites: the main Galicos River Wastewater Treatment Project site and fields in the Axios Delta. These two sites were a distance of 10 km apart from each other (Map 3.1).

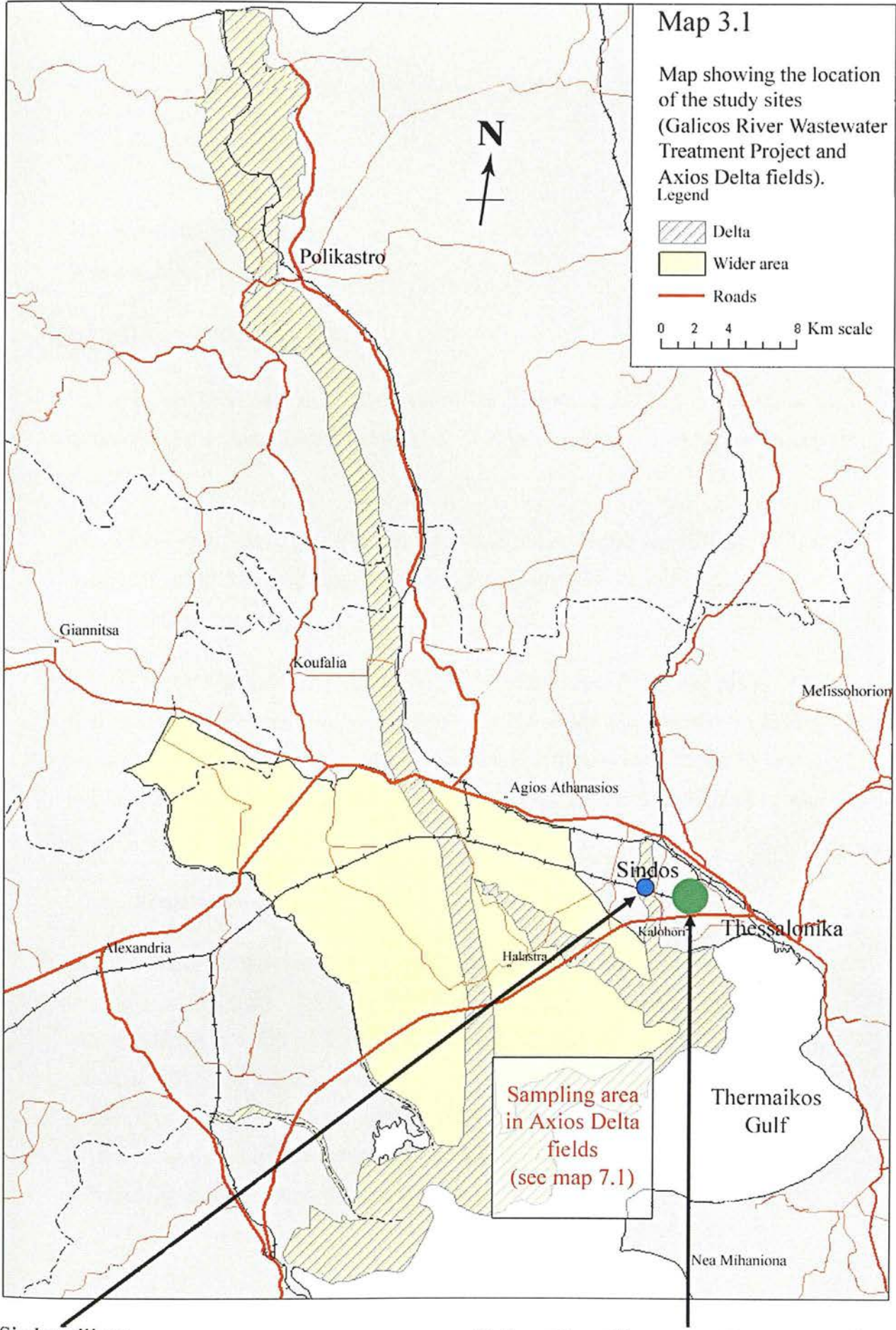
3.2 Soil analysis

3.2.1 Routine storage and analysis

The fresh moist loose soil samples and intact soil cores were always stored for as little time as possible at 4 °C prior to analysis (Breitenbeck and Bremner, 1987). Where air-dry soil samples were required, the soil was placed in trays, air dried at room temperature, passed through a 2 mm sieve and stored in plastic bags until required. Where analysis on fresh moist soil was required, the samples were mixed thoroughly before subsampling for analysis.

3.2.2 Determination of soil moisture content

The method described by Gardner (1986) was used to determine gravimetric soil moisture content. An aluminium cup was weighed. Approximately 25 g of fresh soil was put into the cup and weighed again. The increase in weight, which represented the weight of fresh soil, was calculated (W_1). The cup containing fresh soil was then placed in an oven overnight at 105 °C. The sample was removed from the oven and cooled in a desiccator containing an active desiccant and weighed again. The weight of oven-dry soil was calculated (W_2). The loss in weight gave the amount of water held by the soil.



Calculation:

$$\% \text{ moisture} = \frac{W_1 - W_2}{W_2} \times 100 \quad (\text{Equation 3.1})$$

where

W_1 = weight of fresh soil and

W_2 = weight of oven-dry soil

3.2.3 Determination of soil pH

Soil pH was measured in a 1:2.5 suspension of soil in distilled water using a combination pH glass electrode (Model 8172 Ross Sure-Flow combination) (Black *et al.*, 1965).

Air-dry soil samples (20 g) were transferred into 100 ml beakers and 50 ml distilled water was added. The soil suspension was stirred several times with a glass rod and left to stand for 2 hours.

The pH meter was calibrated using two buffer solutions of pH 7 and pH 4. The calibrated electrode was then rinsed with distilled water and placed in a beaker containing the soil sample suspension. The sample suspension was stirred by gently swirling the electrode. When the display was stable, the sample pH reading was recorded.

3.2.4 Preparation of saturated soil paste extract

Each saturated soil paste extract was prepared according to the procedure described by Bower *et al.* (1952). A known weight of air-dry soil of known moisture content was transferred to a 500 ml beaker. The weight of the beaker and its contents was recorded. Distilled water was added to the soil and mixed with a spatula until a condition of saturation was reached. At saturation, the soil mass was plastic enough to flow slightly when the container was tipped; the soil surface glistened as it reflected light and all the air was displaced. The beaker and its contents were

reweighed and the weight recorded again. The increase in weight was calculated, indicating the weight of water added. Saturation moisture percentage was calculated from the weight of oven dry soil and the sum of the weight of water added and that originally present in the air-dry sample.

Calculation:

$$\text{Wt.oven- dry soil} = \frac{\text{wt. air- dry soil} \times 100}{100 + \% \text{ air- dry moisture}} \quad (\text{Equation 3.2})$$

$$\text{Total water} = \text{water added} + (\text{wt.air-dry soil} - \text{wt. oven-dry soil}) \quad (\text{Equation 3.3})$$

$$\text{Saturation percentage} = \frac{\text{Total water} \times 100}{\text{Oven - dry soil weight}} \quad (\text{Equation 3.4})$$

The saturation soil paste was allowed to stand overnight following preparation, then it was transferred to a Buchner funnel fitted with a filter paper and was filtered by suction using a suction pump. The extract samples thus obtained were measured for electrical conductivity (EC_e), soluble cations, chloride concentration and for water-soluble organic carbon (WSOC).

3.2.4.1 Determination of electrical conductivity ($dS\ m^{-1}$)

The total concentration of the ionized constituents in the saturated soil extract was measured using a Wheatstone bridge as described by Richards (1954). The bridge conductivity cell was filled with 0.01M potassium chloride solution as the standard reference solution (0.746 g potassium chloride was dissolved in a 1 litre volumetric flask, made up to the mark using distilled water and mixed thoroughly). 0.01M potassium chloride solution has an electrical conductivity of $1.412\ dS\ m^{-1}$ at $25\ ^\circ C$. The temperature of the saturated soil extract sample was recorded (t). The bridge conductivity cell was rinsed using distilled water and filled with the saturated soil extract sample and its electrical conductivity at the recorded temperature was measured (EC_t). The electrical conductivity of the saturated soil extract sample at $25\ ^\circ C$ was calculated. The bridge conductivity cell was kept filled with distilled water when it was not in use.

Calculation:

$$EC_{25} = EC_t \times f_t \quad (\text{Equation 3.5})$$

where

$$EC_{25} = EC \text{ at } 25^\circ\text{C}$$

EC_t = EC at the temperature of the soil extract sample.

f_t = Temperature conversion factor to the standard temperature of 25°C .

3.2.4.2 Determination of soluble cations (mg l^{-1})

The concentrations of soluble cations (Na^+ , K^+ , Ca^{++} , Mg^{++}) in saturation extracts were measured using Inductively Coupled Plasma Analytical Atomic Spectrometry (ICPAASTTM 6IE Spectrometer).

Prior to measuring the concentrations of soluble cations in the saturation extracts, standard solutions of Na, K, Ca and Mg (1 and 10 mg l^{-1}) were prepared using ICP reagent-grade standards of 100 and 1000 mg l^{-1} . The spectrometer controls were adjusted to read the standard solutions for each cation as recommended by the manufacturer. Blanks were also read. Then, a sequence of extract samples was automatically introduced into the system. The concentration of the specific cation in the sample solution expressed in mg l^{-1} was measured and printed out. Where soluble cation concentrations in saturation extracts were above the top standard, samples were diluted with distilled water and re-analysed.

The concentration of soluble cations as $\text{mmol}_c \text{ } 100 \text{ g}^{-1}$ of soil was calculated using the following formula:

Soluble cation in $\text{mmol}_c \text{ } 100 \text{ g}^{-1}$ soil =

$$(\text{mmol}_c \text{ l}^{-1} \text{ of soluble cation in saturation extract}) \times \left\{ \frac{\text{saturation percentage}}{1000} \right\} \quad (\text{Equation 3.6})$$

3.2.4.3 Determination of carbonate and bicarbonate (mg l^{-1})

Carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) concentrations were determined using the methods described by Hesse (1971).

Air-dry soil samples (20 g) were shaken with 100 ml distilled water for one hour in a shaking machine, and filtered using Whatman No.42 filter paper. A 5 ml aliquot from the soil-water extract was pipetted out onto a white porcelain basin. A few drops of 1 % phenolphthalein indicator were added. The 1 % phenolphthalein indicator was prepared by dissolving 1 g phenolphthalein powder in 100 ml of 60 % alcohol. At this point the solution did not turn pink. This indicated that there was no carbonate in the soil samples.

5 drops of 0.01 % methyl orange ($0.01 \text{ g } 100 \text{ ml}^{-1}$ distilled water) were added to the same solution used to measure CO_3^{2-} . The solution was titrated with 0.005 M sulphuric acid to a light colour end point. The volume of sulphuric acid used was recorded (Z ml). A blank determination was run using distilled water. The volume of sulphuric acid used in the blank solution titration was recorded (Y ml).

Calculation:

$$\text{HCO}_3^- (\text{mmol l}^{-1}) = \frac{(Z - Y) \times M \times 1000}{\text{aliquot (ml)}} \quad (\text{Equation 3.7})$$

where

Z= volume in ml of 0.005M sulphuric acid used to titrate sample solution

Y= volume in ml of 0.005M sulphuric acid used to titrate blank solution

M= is the concentration of the sulphuric acid (mol l^{-1})

3.2.4.4 Determination of chloride concentration (mg l^{-1})

The chloride concentration in saturated soil extracts was measured using a potentiometric method based on an ion selective electrode (Adriano and Doner, 1982), in which the concentration of chloride is related logarithmically to its mV reading. A 5 M sodium nitrate solution was prepared as follows: 42.495 g sodium

nitrate (NaNO_3) was dissolved in 50 ml distilled water, transferred into a 100 ml volumetric flask, made up to the mark with distilled water and mixed thoroughly. The outer chamber of the reference was filled with this solution.

Total ionic strength adjustment buffer (TISAB) solution was replaced by C.I.S.A buffer solution: 15.09 g of sodium bromate (NaBrO_3) was dissolved in 500 ml distilled water. 62.5 ml of concentrated nitric acid was added carefully and cooled. The cooled solution was quantitatively transferred to a 1 litre volumetric flask and made up to the mark with distilled water).

A calibration curve was prepared using standard solutions of 1, 5, 10, 50 and 100 $\mu\text{g Cl}^- \text{ml}^{-1}$ by serial dilution of the 1000 $\mu\text{g ml}^{-1}$ stock solution made up from sodium chloride (2.5 g sodium chloride was oven dried for two hours at 105 °C and cooled in a desiccator. 2.103 g of the dried salt was dissolved in distilled water, transferred into a 1 litre volumetric flask and made up to the mark with distilled water).

The pH meter was set up with the Cl^- electrode and a reference electrode and was allowed to warm up for 30 minutes. The correct functioning of the pH meter and the electrodes were checked according to the procedure outlined in the manufacturer's instruction manual.

A 15 ml aliquot from a saturated soil paste extract was pipetted out into a 50 ml plastic beaker. 15 ml of C.I.S.A buffer solution was added. The mixture was allowed to stand for 40 minutes in a fume cupboard to allow the oxidation of Br^- ions. Standards were prepared in the same way: 15 ml aliquot of each standard solution was mixed with 15 ml of C.I.S.A buffer solution and allowed to stand for 40 minutes. C.I.S.A buffer solution was added to give ionic balance to standards and samples. This gives results in terms of concentration (not activity).

After 40 minutes, a magnetic stirring bar was added to each beaker, which was then placed on a magnetic stirrer. The electrode was rinsed with distilled water, dried with tissue paper, immersed in the solution and mV readings were taken after 50 seconds.

An Excel spreadsheet was used to obtain linear coefficients after mV readings of standards were plotted against log Cl^- concentrations, and to provide results in anti log form.

3.2.4.5 Determination of water-soluble organic carbon (WSOC) ($\mu\text{g C g}^{-1}$ dry soil)

Saturated soil paste extracts were analysed for water-soluble organic carbon (WSOC) on a Dohrmann DC-80 TOC Analyser (Sartec Limited, Borough Green, Kent).

The instrument was warmed up for approximately 10 minutes, after the detector had been on for two or more hours. It was ensured that the carrier gas flow at the detector “out” port was set to $180\text{--}220 \text{ ml min}^{-1}$. The total organic carbon/purgeable organic carbon (TOC/POC) mode was turned to the “TOC” position, the detector/output was turned to the “ppm” setting, and the reactor was filled with 2% potassium persulphate reagent (20 g potassium persulphate ($\text{K}_2\text{S}_2\text{O}_8$) was dissolved in a 1 litre volumetric flask. 1 ml of concentrated phosphoric acid was added, made up to the mark with distilled water and mixed thoroughly). The solution was stored in a cool dark place.

A 2000 mg l^{-1} C stock standard solution was prepared (425 mg potassium hydrogen phthalate ($\text{C}_8\text{H}_5\text{O}_4\text{K}$), KHP, was dried to constant weight and transferred quantitatively with a stream of water to a 100 ml volumetric flask and dissolved. 0.1 ml concentrated phosphoric acid was added, and the solution was made up to the mark with distilled water and mixed well). The stock standard solution was stored in a dark glass bottle with refrigeration.

A 400 mg l^{-1} C standard solution was prepared (20 ml from 2000 mg l^{-1} C stock solution was pipetted into a 100 ml volumetric flask and made up to the mark using distilled water). A small amount of the 400 mg l^{-1} C standard solution was put into a sample cup, acidified with 1 drop of orthophosphoric acid and sparged with a gas stream passed through it, using the external sparging device provided with the Dohrmann DC-80 TOC Analyser, for 5 minutes, to remove CO_2 from solution.

A 200 µl volume of the acidified and sparged 400 mg l⁻¹ C standard solution was injected manually by syringe through the injection port to calibrate the instrument. This was a suitable volume, according to the instrument manual, to set up the operating range (10-800 ppm).

Prior to analysis, soil water extracts were transferred to sample cups, acidified with 1 drop of orthophosphoric acid to remove inorganic carbon, and sparged for 5 minutes. 200-µl volumes of the acidified and sparged samples were injected manually by syringe through the injection port and analysed for WSOC.

Distilled water, used in the saturated soil paste preparation, was filtered through filter paper, acidified, sparged in the same way and analysed for water-soluble organic carbon to act as a blank.

Calculation:

Adjusted WSOC in soil extract (mg l⁻¹) = WSOC (mg l⁻¹) in soil extract – WSOC in blank sample (mg l⁻¹). (Equation 3.8)

WSOC (mg kg⁻¹) in soil sample = $\frac{\text{adjusted WSOC (mg l}^{-1}\text{) in soil extract}}{\text{dilution factor}}$ (Equation 3.9)

Since we used saturated soil paste extract, $\frac{\text{WSOC (mg kg}^{-1}\text{) in soil sample}}{\text{WSOC (mg l}^{-1}\text{) in soil extract}} = 1$

The amount of WSOC was corrected on a soil oven-dry basis

3.2.5 Determination of exchangeable cations

Soil exchangeable cations ((Na⁺, K⁺, Ca⁺⁺, Mg⁺⁺) were extracted with a neutral 1 M ammonium acetate solution as outlined in the method described by Bower *et al.* (1952), and the concentrations of the various cations expressed in mg l⁻¹ were measured using the ICPAASTM 6IE Spectrometer.

Three replicates of 5 g air-dry soil samples (<2.00 mm) of known moisture content were transferred into 50 ml centrifuge tubes. 33 ml of neutral 1 M ammonium

acetate solution was added to each tube. The tube was stoppered and the mixture was shaken for 5 minutes in a shaking machine. The stopper was removed and the tube was centrifuged at 1500-2000 rpm for about 5 minutes until the supernatant liquid was clear. The supernatant liquid was decanted completely into a 100 ml volumetric flask. The sample was extracted with ammonium acetate solution two more times using this procedure, and the supernatant liquid was decanted into the same flask, made up to the mark with neutral 1 M ammonium acetate solution and mixed well.

Prior to measuring the concentrations of Na^+ , K^+ , Ca^{++} , and Mg^{++} cations in the soil extracts, standard solutions of Na, K, Ca and Mg were prepared as described above in section 3.2.4.2. These stock standards were prepared with neutral, 1 M ammonium acetate solution. Standards and soil extracts were then loaded into the automatic sampler. The concentrations of the specific extractable cation in the sample solutions were measured as mg l^{-1} and the results were printed out. Where extractable cation concentrations were greater than the top standard concentration, samples were diluted with neutral 1 M ammonium acetate solution and re-analysed. The concentrations of the cations (Na^+ , K^+ , Ca^{++} , Mg^{++}) in the 1 M ammonium acetate solution were measured and used as blank values which were subtracted from the concentrations of cations measured in the soil sample extracts.

Calculation:

The $\text{mmol}_c \text{ 100 g}^{-1}$ soil exchangeable cation was calculated as follows:

$$\text{mmol}_c \text{ 100 g}^{-1} \text{ soil extractable cation} = \left\{ \frac{\text{mg kg}^{-1} \text{ soil}}{10 \times \text{molecular weight}} \right\} \text{ (Equation 3.10)}$$

$$\text{mg kg}^{-1} \text{ in soil} = \text{mg l}^{-1} \text{ in soil extract} \times \text{dilution factor}$$

Since 5 g of soil was extracted and made up to 100 ml in volume, the dilution factor was 20.

While, mg kg^{-1} of each cation in soil = (Exchangeable + water soluble)

$$\text{mmol}_c \text{ 100 g}^{-1} \text{ soil exchangeable cation} = (\text{mmol}_c \text{ 100 g}^{-1} \text{ soil extractable cation}) - (\text{mmol}_c \text{ 100 g}^{-1} \text{ soil soluble cation in saturated soil extract})$$

3.2.6 Determination of cation exchange capacity

The cation exchange capacity (CEC) was determined based on the measurement of the total quantity of negative charges per unit weight of the soil, by the replacement of adsorbed sodium from the soil sample by extraction with neutral 1 M ammonium acetate solution (Bower *et al.*, 1952). The sodium concentration in the ammonium acetate extracts as mg Na l^{-1} was measured using the ICPAASTM 6IE spectrometer and the mmol_e of sodium adsorbed by 100 g of oven-dry soil was calculated. These values were reported as cation exchange capacity.

Replicate 5 g air-dry soil samples (<2.00 mm) of known moisture content were transferred into 50 ml centrifuge tubes. 33 ml of 1 M sodium acetate solution (pH 8.2) was added to the soil. Sodium acetate solution was used because the solubility of CaCO_3 in 1 M sodium acetate of pH 8.2 is much lower than it is in neutral, 1 M ammonium acetate solution. Bower *et al.* (1952) observed that the CEC of soils by the sodium acetate method was unaffected by the presence of CaCO_3 .

The tubes were stoppered and the mixtures were shaken for 5 minutes in a shaking machine. The stoppers were removed and the tubes were centrifuged at 1500-2000 rpm for about 5 minutes until the supernatant liquid was clear. The supernatant liquid was decanted and discarded. Samples were treated in this manner with 33 ml portions of 1 M sodium acetate solution a total of 3 times and the supernatant liquid was discarded each time.

33 ml of 95 % ethanol was added to each tube containing the soil sample. The mixture was shaken, centrifuged at 2000 rpm for 5 minutes and the supernatant liquid was decanted and discarded. Samples were washed with 95 % ethanol two more times. Finally, the same soil samples were extracted with three portions of 33 ml 1 M ammonium acetate solution. To replace the adsorbed sodium from the soil sample each time, the mixture was shaken, centrifuged, and the supernatant liquid was collected in a 100 ml volumetric flask. The combined extract was diluted to 100 ml with neutral, 1 M ammonium acetate solution and mixed well. The sodium concentration in the combined extract was then measured.

Prior to measuring the sodium concentration in the combined extract, standard solutions of 0, 5, 10, 20, 30, 40, 50 and 100 mg Na l⁻¹ were prepared by serial dilution from a Na stock solution of 1000 mg Na l⁻¹. Na stock solution was prepared by dissolving 2.541 g of sodium chloride in 1 litre of 1 M ammonium acetate solution. The standard solutions and the extracts were then measured for Na in the ICPAASTM 6IE spectrometer and the results were printed out and expressed in mg l⁻¹. Where Na concentrations were greater than the top standard, samples were diluted with neutral, 1 M ammonium acetate solution and re-analysed as before.

Calculation:

$$\text{CEC as mmol}_e \text{ 100 g}^{-1} \text{ soil} = \frac{10 \times \text{Na concentration (mmol}_e \text{ l}^{-1})}{\text{Oven dry weight of soil sample}} \quad (\text{Equation 3.11})$$

3.2.7 Determination of particle size distribution

Mechanical analysis for sand, silt and clay particles was carried out using a standard hydrometer method, ASTM no.152, as described by Black *et al.* (1965).

Air-dry soil samples (50 g, <2.00 mm) of known moisture content were weighed in a 200 ml beaker. Each soil sample was wetted by means of a small amount of distilled water. 20 ml of 5 % calgon solution (50 g calgon (Na-hexa-metaphosphate) l⁻¹ distilled water) was added to the soil sample. The contents were mixed, covered with a watch glass and kept overnight. The contents were quantitatively transferred into a dispersion cup using a stream of distilled water and mixed in an electrical mixer for 5 minutes. The suspension was transferred again into a 1 litre sedimentation cylinder with the help of a stream of water and made up to the mark with distilled water.

The suspension was allowed time to equilibrate thermally, then its temperature was recorded. A plunger was inserted, and moved up and down to mix the contents thoroughly for 1 minute, carefully holding the bottom of the cylinder to prevent tipping. The stirring was finished with two or three slow, smooth strokes. The

plunger was moved, tipped slightly to remove adhering drops and the time was recorded immediately. A drop of amyl alcohol was added to disperse the foam.

After 20 seconds, the hydrometer was carefully lowered into the suspension and the reading of its scale at the top of the meniscus was taken after exactly 40 seconds. This reading gave the amount of silt and clay. Care was taken to ensure that the hydrometer was kept away from the cylinder wall. The hydrometer was then removed, rinsed and dried with tissue paper.

The suspension was allowed to stand for 2 hours (including the 40 seconds reading) without mixing, then the hydrometer was again inserted very carefully and its reading was recorded followed by a temperature reading. This reading gave the amount of clay present.

The hydrometer type used (ASTM no.152) was calibrated at 19.5 °C. Therefore, a temperature correction of 0.3 graduation on the hydrometer for every 1 °C above or below 19.5 °C was applied. The temperature correction was added to the hydrometer reading when the recorded temperature was above 19.5 °C and subtracted when it was below 19.5 °C. The corrected hydrometer reading at each time was calculated.

Calculation:

The first reading (at 40 seconds) gave the amount of silt and clay. The second reading (after 2 hours time) gave the amount of clay. The amounts were calculated as follows.

$$\% (\text{silt} + \text{clay}) = \frac{\text{Corrected hydrometer reading (40 seconds)}}{\text{Oven - dry soil weight (g)}} \quad (\text{Equation 3.12})$$

$$\% \text{ clay} = \frac{\text{Corrected hydrometer reading (2 hours)}}{\text{Oven - dry soil weight (g)}} \quad (\text{Equation 3.13})$$

$$\% \text{ silt} = \% (\text{silt} + \text{clay}) - \% \text{ clay}$$

$$\% \text{ sand} = 100 - \% (\text{silt} + \text{clay})$$

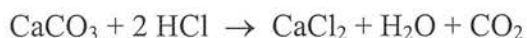
3.2.8 Determination of the carbonate content of soils

The calcium carbonate content was determined using the method described by Rowell (1994).

Air-dried and milled soil samples (10 g) of known moisture content were transferred into 250 ml conical flasks. 20 ml of 2 M hydrochloric acid was added to the soil in each flask and the flasks were allowed to stand until the reaction was complete. A complete reaction was ensured by boiling gently for 10 minutes. The suspension was quantitatively transferred to a 100 ml volumetric flask through a funnel and filter paper (Whatman No.1), washed with distilled water, made up to the mark, and mixed thoroughly.

10 ml of the sample solution was pipetted into a 250 ml conical flask. 50 ml of distilled water and a few drops of phenolphthalein indicator were also added. The solution was titrated with 0.1M sodium hydroxide using a micro-burette to a pink colour end point.

A 2 M hydrochloric acid solution was standardized using a micro-burette against the 0.1 M sodium hydroxide solution, using different volumes of the hydrochloric acid. The amount of calcium carbonate content was calculated assuming that 2 moles of HCl would react with 100.1 g CaCO₃ (Rowell, 1994).



Calcium carbonate content was expressed as a percentage of the mass of oven dry soil.

3.2.9 Determination of soil organic carbon (mg C g⁻¹ dry soil)

Soil organic carbon was determined using a complete wet oxidation method involving an excess of a mixture of concentrated sulphuric acid and aqueous potassium dichromate as an oxidising agent, with external heating (Rowell, 1994). Prior to starting the analysis, the following reagents were prepared:

a) 66.7 mM potassium dichromate: 45 g potassium dichromate ($K_2Cr_2O_7$) was ground to a powder, oven dried overnight at $105^\circ C$ and cooled in a desiccator. 39.23 g of the dried salt was dissolved in approximately 700 ml of distilled water. 800 ml of 98 % sulphuric acid was added and the mixture was allowed to cool. 400 ml of 85 % orthophosphoric acid was added and stirred in until all the solids were dissolved; the mixture was then cooled. The solution was diluted to 2 litres with distilled water.

b) 0.4 M ferrous ammonium sulphate: this was prepared by dissolving 230 g ammonium ferrous sulphate ($(NH_4)_2 SO_4 FeSO_4.6H_2O$) in a mixture of 5 ml of 98 % sulphuric acid and 1.5 litres of distilled water. The solution was diluted to 2 litres with distilled water.

c) Barium diphenylamine sulphonate indicator: 0.2 g barium diphenylamine sulphonate was dissolved in approximately 150 ml warm water in a 200 ml volumetric flask. 20 g barium chloride ($BaCl_2.2H_2O$) was added. The mixture was warmed up in order to dissolve. It was then cooled, diluted to 200 ml with distilled water, mixed thoroughly, left to stand overnight and then filtered.

Air-dry and milled ground soil (1 g) of known moisture content, was transferred into a digestion flask. 40 ml of 66.7 mM potassium dichromate was added to the soil and digested at $130-135^\circ C$ for 2 hours. The cooled digest was quantitatively transferred to a 100 ml conical flask. 2 ml of barium diphenylamine sulphonate indicator was added to the solution. The solution was titrated with 0.4 M ferrous ammonium sulphate solution using a micro-burette to a bright green colour end point. The volume of ferrous ammonium sulphate solution used was recorded (X ml).

0.4 M ferrous ammonium sulphate ($(NH_4)_2SO_4.FeSO_4.6H_2O$) was standardized using a micro-burette against the 66.7 mM potassium dichromate solution as follows: 40 ml of 66.7 mM potassium dichromate solution was transferred into a digestion flask, and digested and titrated against 0.4 M ferrous ammonium sulphate in the same way as above. The volume of ferrous ammonium sulphate solution used in the titration of potassium dichromate solution was recorded (Y ml).

Calculation:

$$\text{Carbon content (mg C g}^{-1}\text{ air-dry soil)} = 48 \times \left\{ \frac{(1 - X/Y)}{\text{Soil sample weight (g)}} \right\} \quad (\text{Equation 3.14})$$

The carbon content was corrected to a soil oven dry weight basis.

3.2.10 Determination of mineral nitrogen ($\mu\text{g N g}^{-1}$ dry soil)

Mineral N, that is ammonium-N ($\text{NH}_4\text{-N}$) and nitrate-N ($\text{NO}_3\text{-N}$), were determined using the method described by Bremner (1965). Freshly sampled soil samples (20 g) of known moisture content were shaken with 100 ml 1M KCl extracting solution for about 1 hour, filtered using Whatman No. 42 filter paper, and analysed for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ by continuous flow analysis on a Chemlab Instruments Autoanalyser. Peaks were analysed using the Kontron integrator package on a PC.

The ammonium-N determination was based on the Bertholet reaction in which alkaline phenol and hypochlorite react with ammonia to form a blue indophenol (Davidson *et al.*, 1970). Unfortunately, both of these reagents have limited stabilities and are unpleasant to handle. The present method uses a modification of the Bertholet reaction in which a similar overall reaction takes place, but substitutes salicylate (2-hydroxybenzoate) for phenol, and dichloroisocyanurate (D.I.C) for hypochlorite. The D.I.C decomposes in an alkaline solution to form hypochlorite ions. Nitroprusside was added as a catalyst and the colour intensity was measured at 650 nm wavelength. The analytical grade reagents used in ammonium-N determination are shown in Table 3.1.

A continuous flow analysis system was also employed in the nitrate-N determination using hydrazine and copper as reducing agent. The colour intensity was measured at 520 nm. The analytical grade reagents used in nitrate-N determination are listed in Table 3.2.

Standard solutions of 0.5, 1, 2, 3, 4 and 5 mg N l^{-1} ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) were prepared by serial dilution from a stock standard solution of 1000 mg N l^{-1} prepared

from ammonium chloride, and a stock standard solution of 200 mg N l^{-1} prepared from potassium nitrate. 3.817 g ammonium chloride (NH_4Cl) was dissolved in a 1 litre volumetric flask, made up to the mark with distilled water and mixed thoroughly. 1.444 g potassium nitrate was dissolved in a 1 litre volumetric flask, made up to the mark with distilled water and mixed well.

The standard solutions were transferred to sample cups and placed on the sampler in order of increasing concentration. An aliquot of 1 M KCl extracting solution was filtered through Whatman No. 42 filter paper, put in a sample cup, placed on the sampler, and measured as a blank.

The assay was run using a sample time of 50 seconds and a wash time of 50 seconds. The amounts of ammonium-N and nitrate-N were expressed on a soil oven-dry weight basis.

Table 3.1 The analytical grade reagents used for ammonium-N determination.

Reagent	Amount
<u>1. Salicylate</u>	
Sodium salicylate (2-hydroxybenzoic acid sodium salt)	34 g
Sodium nitroprusside ($\text{Na}_2(\text{Fe}^{\text{III}}(\text{CN})_5\text{NO}) \cdot 2\text{H}_2\text{O}$)	0.40 g
Water	to 1 litre
<u>2. Dichloroisocyanurate (D.I.C)</u>	
Sodium dichloroisocyanurate (dichloro-1,3,5-triazine-2,4,6-trione sodium salt)	0.80 g
Sodium hydroxide (NaOH)	10 g
Water	to 1 litre

Table 3.2 The analytical grade reagents used for nitrate-N determination.

Reagent	Amount
<u>1. 0.4 M Sodium hydroxide</u>	
Sodium hydroxide (NaOH)	16 g
Brij-35 (30 % w/v, GPR)	1 ml
Water	to 1 litre
<u>2. 0.2 M Sodium hydroxide</u>	
Sodium hydroxide (NaOH)	8 g
Brij-35 (30 % w/v, GPR)	1 ml
Water	to 1 litre
<u>3. Stock Copper Sulphate Reagent</u>	
Cupric sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)	4 g
Water	to 1 litre
<u>4. Hydrazine-Copper Reagent</u>	
Hydrazine sulphate	1.5 g
Stock copper sulphate reagent	3 ml
Water	to 1 litre
Hydrazine sulphate was dissolved in approximately 300 ml distilled water in a 1 litre volumetric flask. Stock copper sulphate reagent was added, made up to the mark and mixed thoroughly.	
<u>5. Sulphanilamide reagent</u>	
Sulphanilamide	10 g
Concentrated phosphoric acid	100 ml
N-1-naphthylethylenediamine dihydrochloride	0.5 g
Water	to 1 litre
Phosphoric acid was added to approximately 500 ml distilled water in a 1-litre volumetric flask and mixed. The sulphanilamide and the naphthylethylenediamine dihydrochloride were added to the mixture, stirred until dissolved, made up to the mark and stored in a brown bottle.	

3.2.11 Determination of total nitrogen

Soil samples were analyzed for total nitrogen using the modified Kjeldahl method supplied by Büchi Laboratories, Switzerland (Büchi Nitrogen - Information leaflet No. 1). Digestion of 500 mg of ball-milled soil samples with 20 ml concentrated H_2SO_4 and 6 tablets of copper catalyst was carried out in glass digestion tubes in a digestion unit for one hour. The copper catalyst was in the form of commercial catalyst manufactured by VWR International (Ltd), where each tablet contains 1 g sodium sulphate and 0.1 g copper (II) sulphate. The tubes were cooled to approximately room temperature, connected to the distillation unit and diluted with 50 ml distilled water. Boric acid distillation receiver, which was prepared from 60 ml of 2 % boric acid (2 g 100 ml^{-1} distilled water) plus 5 drops of Sher indicator was transferred to a 250 ml conical flask and placed below the still outlet. Sher indicator was prepared by mixing 0.35 g of bromocresol green dissolved in 10 ml of alcohol, 10 ml of 0.1 M sodium hydroxide, 200 ml of distilled water, and 0.75 g p-nitrophenol dissolved in 5 ml alcohol in a 250 ml volumetric flask. The mixture in the volumetric flask was made up to the mark with distilled water (Büchi Nitrogen – Information leaflet No. 1). The diluted sample was neutralized by the addition of 40 % sodium hydroxide (40 g 100 ml^{-1} distilled water) until the sample showed a distinct change of colour to brown. Distillation was started after neutralization. After the distillation was complete, the ammonia in the distillate receiver was titrated directly with 0.25 M H_2SO_4 in a micro-burette to the end point. The volume of H_2SO_4 used in the sample titration was recorded (V_1).

The digestion products of the reagents without a soil sample and distilled as above were run as a blank. The blank distillate solution was then titrated with 0.25M H_2SO_4 , and the volume of H_2SO_4 used in the blank titration was recorded (V_2).

Calculation:

$$\text{Total N}_2 (\%) = \frac{(V_1 - V_2) \times M \times f}{\Sigma(\text{mg})} \times 1400 \quad (\text{Equation 3.15})$$

Where

V_1 is the sample titre volume of H_2SO_4
 V_2 is the blank titre volume of H_2SO_4
 M is the molarity of H_2SO_4
 f is the acid factor
 Σ (mg) is the weight of soil sample in mg

3.2.12 Soil strength measurements

Measurement of soil strength (the resistance of a soil to penetration) was carried out on repacked and intact cores using a drop cone penetrometer according to the method described by Bradford (1986).

The cone penetrometer is a simple versatile device, which is widely used to check the strength of the soil in terms of its resistance to the penetration of a standard cone. The soil penetration resistance is a function of soil moisture content, soil specific weight and soil type. The soil type is characterized by means of a clay ratio, which is the ratio of the clay content of the soil to the contents of silt and sand. The cone penetration resistance has been widely used to predict tractive capabilities of off-road vehicles (Dwyer *et al.*, 1974), to predict draught forces in tillage operation (Gill and Vanden Berg, 1967), and to assess compaction related to vehicle traffic (Raghavan and McKyes, 1977).

Repacked and intact soil cores confined in metal rings were placed on the base plate of the drop cone penetrometer. The tip of the cone was positioned at the soil surface very accurately using coarse and fine adjustment wheels. A reading of 0.1 mm on a dial gauge corresponded exactly to the position of the cone on the soil surface. A cone cross-sectional area of 0.1099 cm^2 was used.

When the tip of the cone just touched the surface of the soil core, a clockwork mechanism was hand-operated, whereby on touching a release button, the shaft was released for 5 seconds and then clamped rigidly again. The depth of penetration of the cone in the soil during these 5 seconds was recorded from the dial gauge reading (mm). This process was repeated at six points on each soil core. The cone was wiped clean between measurements.

Each dial reading (mm) was converted into a penetration force in kilograms (kg) using the calibration chart provided. The penetration resistance at each point on the core was calculated and from these values, the average penetration resistance for each core was calculated to obtain a reliable estimation.

Calculation:

$$\text{Penetration resistance (kg cm}^{-2}\text{)} = \left\{ \frac{\text{Penetration force (kg)}}{\text{Conc cross-sectional area}} \right\} \quad (\text{Equation 3.16})$$

$$\text{Penetration resistance (kN cm}^{-2}\text{)} =$$

$$\text{Penetration resistance (kg cm}^{-2}\text{)} \times 98.07 \quad (\text{Equation 3.17})$$

3.3 Soil water measurements

3.3.1 Infiltration rate (cm h⁻¹)

Soil infiltration rate (downward movement of irrigation water or rainfall into the soil surface) was measured by a single ring infiltrometer method described by Bouwer (1986).

A number of methods are used for measuring soil infiltration rate which is described as the time required for a given amount of water to enter the wet portion of a soil column. This can be considered as a transmitting zone through which water is transmitted with little change in water content (Colman and Bodman, 1944). The most commonly used method is a flooding technique. This technique is performed by using either a single or double ring infiltrometer (Bouwer, 1986). In this study, single ring infiltrometers of 3.8 cm diameter and 1 cm height were used for measuring the infiltration rate of soil cores. The main disadvantage of this method is that the infiltration below ring infiltrometers is not vertically downwards but diverges laterally. This lateral divergence can be caused by capillary forces, by layers of reduced hydraulic conductivity deeper in the soil profile or by the pressure head of the water at the soil surface due to the water depth inside the ring infiltrometer. As a result, the infiltration rate inside the infiltrometer will be higher than the true infiltration rate for vertical flow. This problem can be minimized and a

true vertical flow below infiltrometers can be achieved by establishing a good bond between the soil and the ring infiltrometer wall (Bouwer, 1986).

A metal ring (3.8 cm diameter, 1 cm height) used as a single ring infiltrometer was fixed over the surface of the soil core. A known volume of water was applied manually to the small steel ring, and the time required for a given volume of water to enter the surface was measured (Bouwer, 1986).

The continuity of measurements depended on the purpose of the infiltrometer test. When the effect of irrigation water quality on infiltration rate of irrigated soil was studied, the measurements were continued until the infiltration rate became nearly constant. However, when the effect of crust formation on infiltration rate was studied, the measurements were applied until a certain volume of water had passed into the soil.

Calculation:

Infiltration rate was defined as a flux density of water passing through the soil surface (Don Scott, 2000). Thus, mathematically, infiltration rate (I) was defined as:

$$I = \frac{Q}{At} \quad \text{(Equation 3.18)}$$

where

Q is the volume of water infiltrating per unit area of soil surface (A) per unit time (t)
Results were expressed in cm h^{-1} .

3.3.2 Soil moisture release

Soil moisture release was plotted according to the method described by Topp *et al.* (1993).

The standard Reference State for soil water potential is a pool of pure free water at the soil surface, at the same temperature as the soil water and at atmospheric pressure. The total soil water potential Ψ_t , is “the amount of useful work per unit

mass of pure water that must be done by means of externally applied forces to transfer reversibly and isothermally an infinitesimal amount of water from the standard state to the soil liquid phase at the point under consideration” (International Society of Soil Science, 1976). The total soil water potential may be divided into gravitational (Ψ_g), osmotic (Ψ_o) and matric potential (Ψ_m) components. Osmotic potential is due to the presence of solutes, gravitational potential is a function of depth, and matric potential is due to soil matrix and overburden effects. Most apparatus for measuring water potential (e.g. tension tables) involve a measurement of pressure or length. Therefore, potentials are more frequently expressed as energy per unit volume, which has the units of pressure, Pa in SI units, or energy per unit weight of the soil water, which has the units of length (m of water). The soil water content at a potential of -1500 kPa has been widely used as an index of permanent wilting point since it was first suggested by Richards and Weaver in 1943. However, the water potential at field capacity is about -5 kPa.

The water release characteristic (the relationship between the water content of a soil and its matric potential) was found to be an indicator for the ability of the soil to store water that is available to growing plants, and for the aeration status of a drained soil (Townend *et al.*, 1991). Soil water desorption curves are useful directly and indirectly as indicators of other soil behaviour, such as drainage, aeration, infiltration and rooting patterns (Topp *et al.*, 1993). Moreover, it can be used to estimate the pore size distribution (Hillel, 1971).

The total soil water potential is always negative, unless water is poured over a saturated soil. Since the manipulation of negative numbers is sometimes inconvenient, matric suction is defined as the negative of matric potential. As soil becomes drier, the matric potential of the soil decreases (more negative). As a result, not only is water removed from soil pores but also the films of water held around soil particles are reduced in thickness. A number of methods are used for measuring soil moisture release. The soil samples needed to plot the retention curve may be either repacked samples or samples of “natural” structure (Klute, 1986) and many attempts have been made to define adequate and useful mathematical

functions for the desorption curve (Brooks and Corey, 1964; Clapp and Hornberger, 1978). Bruce and Luxmoore (1986) have described several field methods.

The most common items of laboratory apparatus used are the tension plate and the pressure membrane (Klute, 1986; Topp *et al.*, 1993). A tension plate (Topp *et al.*, 1993) with repacked soil cores was used in this research. This is because sufficient intact (undisturbed) soil cores were not available.

The suitable range of soil water potential (tensions) to investigate water release characteristics using tension plate apparatus was reported to be from 0 to -100 kPa (0 to -1 bar) only (Brady, 1974). The main weakness of laboratory methods is that measurements made on core samples in the laboratory, as is usual, may not represent the situation in the field because they do not include the effects of overburden.

A tension table (45.5 cm by 31.0 cm) was prepared, in which silica flour was used as the tension medium, and glass microfibre paper was used as the retaining mesh over drainage channels. The water tension (matric potential) was applied with a hanging water column, lowered to different positions below the table.

Freshly collected loose soil samples were repacked in metal rings using an oedometer at a dry bulk density of 1.3 g cm^{-3} . The resulting repacked soil cores (7.3 cm in diameter, 4.5 cm high) contained the same weight of 244.8 g dry soil. The cores were weighed and then saturated using tap water to prevent dispersion of soil aggregates. Prior to saturation, the soil core was placed over a fine-gauge nylon mesh fabric and secured with an elastic band to prevent soil falling during saturation and during weight checks throughout the experiment. The saturated cores were reweighed and placed together on the tension table at controlled room temperature ($22 \pm 2 \text{ }^{\circ}\text{C}$). The hanging water column was fixed at -1 kPa (-10 cm). Hydraulic contact between the bottom of the soil core and the tension medium was ensured by slightly twisting the core to deform the tension medium to the shape of the soil surface.

The whole assembly was covered to prevent evaporation losses. The weight of the soil cores was checked every 24 hours. At equilibrium, the weights of the cores

were recorded, hydraulic contact was re-established, and the hanging water column was lowered to the next desired matric potential and so on. Individual cores were returned to the same place and orientation on the tension medium and it was ensured that hydraulic contact was re-established.

The above procedure was repeated to give a stepwise decrease in matric potential to -18 kPa (-180 cm). After the final equilibrium point, the fine-gauge nylon mesh, fabric nylon mesh and elastic band were removed from each soil core. Any adhering soil was removed and replaced on the soil core. The fine-gauge nylon mesh fabric cloth and elastic band were freed of soil, which was retained within the soil core. The fine-gauge nylon mesh fabric cloth and elastic band were air-dried and their total weight was recorded.

Soil cores and steel rings were placed in drying tins of known weight and oven dried at 105 °C for 48 hours. Samples were removed from the oven, the lid on each drying tin was replaced, and the cores were cooled in a desiccator and reweighed to a precision of 0.1 g. Each core's steel ring was removed and cleaned to remove all adhering soil, and its weight was recorded.

Calculation:

Oven-dry soil weight (M_d) =

(oven-dry weight of soil core + wt. of metal ring + wt. of tin) - (wt. of metal ring + wt. of tin)

Weight of wet soil at each measured matric potential for each core $M_{(\Psi)} =$

(wt. of wet soil + wt. of metal ring + wt. of fine cloth + wt. of elastic band) - (wt. of metal ring + wt. of fine cloth + wt. of elastic band)

The volumetric water content $\theta_{[\Psi]}$ at each matric potential (Ψ) was calculated as follows

$$\theta_{[\Psi]} = 100 \left\{ \frac{M_{(\Psi)} - M_d}{V_s P_w} \right\} \quad \text{(Equation 3.19)}$$

where

$M_{(\Psi)}$ = weight of soil (and water) measured at $-\Psi$

M_d = weight of oven-dry soil

V_s = volume of soil

P_w = density of water

Soil water release data were presented as graphical relationships between volumetric moisture content and the matric potential with which the water was held, over the range of 0 to -18 kPa (0 to -180 cm).

3.3.3 Saturated hydraulic conductivity (cm h^{-1})

The saturated hydraulic conductivity of the soil samples was measured using the constant head method as described by Klute and Dirksen (1986).

The hydraulic conductivity of a soil is a measure of its ability to transmit water (Klute and Dirksen, 1986). The main practical limitations in measurements of hydraulic conductivity are that (a) large numbers of samples are required because soil hydraulic conductivity is highly variable, and (b) the changes in the concentration and types of cationic species in the soil solution may cause large changes in the hydraulic conductivity of soils containing significant amounts of clay, especially swelling clays (Klute and Dirksen, 1986).

Saturated hydraulic conductivity is one of the soil properties that determine the behaviour of soil water flow systems. It provides a measure of the ability of a saturated porous medium to transmit water (Reynolds, 1993).

Several laboratory methods are used for measuring the hydraulic conductivity of saturated soils. The most commonly used methods are the constant head method and the falling head method. The constant head method described by Klute and Dirksen (1986) was chosen because it is rapid, thus allowing an adequate number of samples to be tested, and both undisturbed cores or repacked soil can be used (Marshall, 1959; Klute and Dirksen, 1986).

A simple piece of apparatus used for measuring the conductivity of saturated samples by the constant head method is shown in Diagram 3.1. Air-dry soil samples

(<2.00 mm) were repacked at a dry bulk density of 1.3 g cm^{-3} in soil leaching columns (3.5 cm in diameter, 17 cm in length). A piece of glass wool was placed at the bottom of the leaching column before the soil was repacked, to prevent soil from entering the column stem. Soil columns were clamped to a support stand. Tap water was passed through the soil columns from a head of water on the soil surface. This head of water was siphoned from a 500 ml volumetric flask filled with tap water, and placed upside down in the top of the soil column using another support stand. The tap water was used to prevent any change in soil structure when it percolated through the soil core.

100 ml beakers were placed under the soil columns. Soil columns were allowed to saturate slowly by downward flow. The volume of percolate that passed through the soil column in 30 minutes and the hydraulic head difference (Δh) were measured. The hydraulic head difference was the vertical distance from the upper water level to the bottom of the soil column (Figure 3.1).

The above procedure was repeated ten times. The saturated hydraulic conductivity (K_s) was calculated every 30 minutes and the average value for each soil column was determined to obtain a reliable estimation.

Calculation:

$$K_s \text{ (cm min}^{-1}\text{)} = \left\{ \frac{VL}{At(\Delta h)} \right\} \quad \text{(Equation 3.20)}$$

where

K_s is the saturated hydraulic conductivity, V is the volume of water (cm^3) that passed through the sample of cross-sectional area A (cm^2) in time t (hour) and Δh is the hydraulic head difference imposed across the sample of length L (cm). Results were expressed in cm h^{-1} .

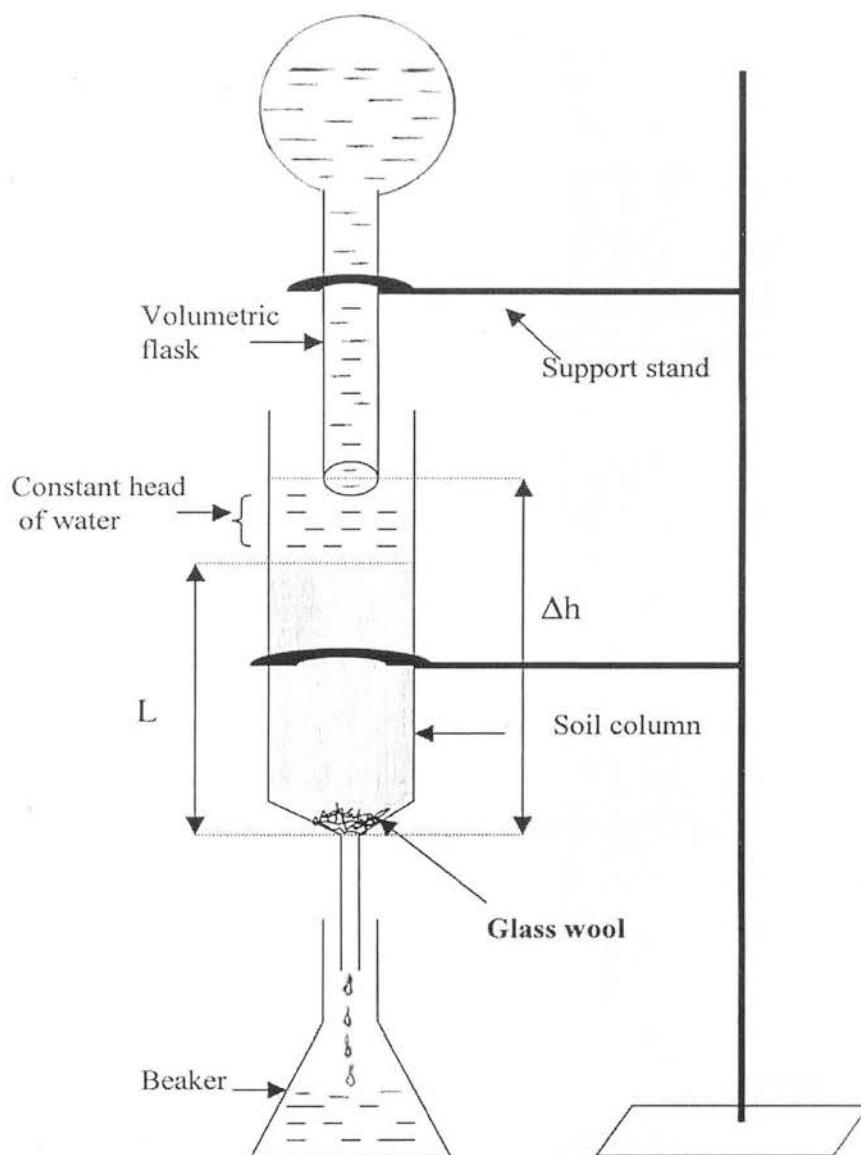


Figure 3.1 A simple apparatus used for measuring saturated hydraulic conductivity of soil samples using the constant head method.

3.4 Methods used for inducing a crust layer on irrigated soil cores

3.4.1 Simulated rainfall

Raindrop impact has been found to be the main factor affecting aggregate disintegration through slaking (Tanaka *et al.*, 1999). When soil particles are broken down by splash or raindrop impact, fine clay grains move into the pore space. As a result, some of the soil pores close. Therefore, the average volume of the total pore space is reduced and clay cement is formed. This cement covers 3-4 mm of soil depth, which forms a hard crust on drying (Dupriez and De Leener, 1992).

Single and multi drop “rainulators” are the main types of rainfall simulators. Single drop rain simulators are used to study the effects of drop size and kinetic energy on crusting and to measure water drop properties (drop size and falling height). However, multi-drop rainulators are used to simulate natural rainfall or field conditions (Nearing and Bradford, 1985). In this study, single drop rainulators were used for inducing a crust layer on repacked and intact irrigated soil cores and uniformity of drop size was achieved using rainulators with protruding hypodermic needles.

Since the drops fall vertically onto the soil surface below, change in rainfall intensity was achieved by controlling the head of water above the level of the drop tubes. Increased uniformity of drop distribution over the soil surface was achieved by rotating the applicator. The falling height in the current study was 0.60 m. This height was found to produce drops with an impact velocity of 3.2 m s^{-1} and a kinetic energy of $3.6 \text{ J mm}^{-1} \text{ m}^{-2}$ (Epema and Riezebos, 1983).

3.5 Gas analysis

3.5.1 Gas chromatograph (GC)

Gas samples were analysed for N_2O and CO_2 on a GC (Hewlett Packard, Model 5890 Series II) connected to an electron capture detector (ECD) for N_2O analysis

and to a thermal conductivity detector (TCD) for CO₂ analysis. GC settings for N₂O and CO₂ analysis are shown in Table 3.3.

All gas samples sent to the ECD for N₂O analysis were backflushed to prevent any gas with a column retention time greater than N₂O from reaching the detector and poisoning it.

3.5.1.1 Measurement of denitrification

Inhibition of N₂O reduction to N₂ by 10 % (by volume) acetylene (C₂H₂) was used to measure total denitrification in laboratory incubations (Klemmedtsson *et al.*, 1977; Yoshinari *et al.*, 1977).

Known gas concentrations of 0.03, 0.10 and 1.02 % CO₂ and 0.33, 1.1, 11, 101.2 and 1006 µg l⁻¹ N₂O were used for GC calibration. A CA-Super-Calc 5.1 spreadsheet was used to obtain linear coefficients of the calibration curve for CO₂ and quadratic coefficients of the calibration curve for N₂O by plotting the logs of their concentrations versus the logs of their peak areas, and taking the antilog of the resulting values. Nitrous oxide and CO₂ concentrations were determined by comparing the peak areas for samples and standards.

3.5.1.2 Automated analysis of gas samples

An autoinjection system for GC analysis of gaseous emissions was used to enable efficient, rapid analysis of multiple gas samples (Arah *et al.*, 1994). The autoinjection system consists of two rotary valves, each with 16 sampling ports. Gas samples were sent to the GC at each sampling time directly from the incubation systems that are linked to the ports by metal tubes.

The autoinjector is computer controlled and can be programmed to sample over any time interval, from any number of ports, and to allow single or multiple sampling. The autoinjector control program also allows automatic backflushing of samples.

The autoinjector allows gas samples to be sent to two different detectors for various gas analyses. Samples are pumped from the ports to a valve, filling two loops. The

pump switches off, and then the valve turns to the analysis position. Carrier gas is then pumped through the loops and the samples are sent for analysis to the GC and relevant detector.

Table 3.3 Conditions for gas chromatographic (GC) analysis of gas samples using a GC connected to an electron capture detector (ECD) for N₂O analysis and a thermal conductivity detector (TCD) for CO₂ analysis.

GC	Type of analysis and Detector	Gas chromatograph parameters
Hewlett Packard Model 5890, Series II	N ₂ O analysis using HP- 5890 Series II GC connected to ECD	Column 5 ft (1.52 m) stainless steel, 1/4" (6.35 mm) o.d., 1.5 mm i.d. HAYSEP Q Column oven temp 60 °C Detector temp 380 °C Carrier gas N ₂ Carrier flow rate N ₂ 40 ml min ⁻¹
	CO ₂ analysis using HP- 5890 Series II GC connected to TCD	Column 9 ft (2.74 m) stainless steel 1/8" (3.17 mm) o.d, 1.5 mm i.d. HAYSEP Q Column oven temp 60 °C Detector temp 100 °C Carrier gas N ₂ Carrier flow rate N ₂ 25ml min ⁻¹

3.5.2 Mass spectrometry

3.5.2.1 General principles

The technique of mass spectrometry determines the isotopic composition of a sample by separating out charged ions on the basis of their mass to charge (m/e) ratio and determining their relative proportions (Robinson and Smith, 1991). In the case of nitrogen analysis this involves the conversion of the N in the sample to N₂. These N molecules are ionised then passed through a magnetic field which separates the N into three components: ¹⁴N₂, ¹⁴N¹⁵N and ¹⁵N₂ with masses of 28, 29 and 30 respectively. The ¹⁵N enrichment of the sample can be determined from the relative

amounts of each component, or more commonly from the ratio of the peaks due to $^{14}\text{N}_2$ and $^{14}\text{N}^{15}\text{N}$ (Hauck, 1982).

$$\text{Atom \% } ^{15}\text{N enrichment} = \frac{100}{2R + 1} \quad (\text{Equation 3.21})$$

where R = the ratio of the peaks due to $^{14}\text{N}_2$ and $^{14}\text{N}^{15}\text{N}$.

3.5.2.2 Experimental procedure

Soil samples were analysed for ^{15}N content using an Integra-CN unit, which involves the coupling of a stable isotope ratio mass spectrometer (IRMS) to an automatic N analyzer (ANA), which converts nitrogen compounds to N_2 by the Dumas oxidation-reduction procedure (Robinson and Smith, 1991).

In the Dumas system (Figure 3.2) the sample is combusted at a very high temperature in a stream of O_2 in an oxidation column packed with NiO . The N in the sample is converted to nitrogen oxides and then reduced to N_2 on a metallic copper column. The tin cup in solid samples acts as a catalyst, raising the temperature to approximately 1700°C to ensure complete combustion. The helium acts as an inert carrier gas to move the N_2 and other combustion products through the system. Water, CO_2 and other contaminants are removed as the N_2 passes over absorbent columns. The N_2 is then ionized and after passing a magnetic field, the ion currents corresponding to the peaks for $^{14}\text{N}_2$, $^{14}\text{N}^{15}\text{N}$ and $^{15}\text{N}_2$ are measured.

3.5.2.3 Soil analysis

Subsamples of ball-milled soil were accurately weighed out into small tin cups and sealed for analysis. Reference values were obtained from standards of known N and ^{15}N content that were analyzed in the same batch as the samples. These standards allow the MS computer software to calibrate the ion beams and current ratios and calculate values for total N and ^{15}N .

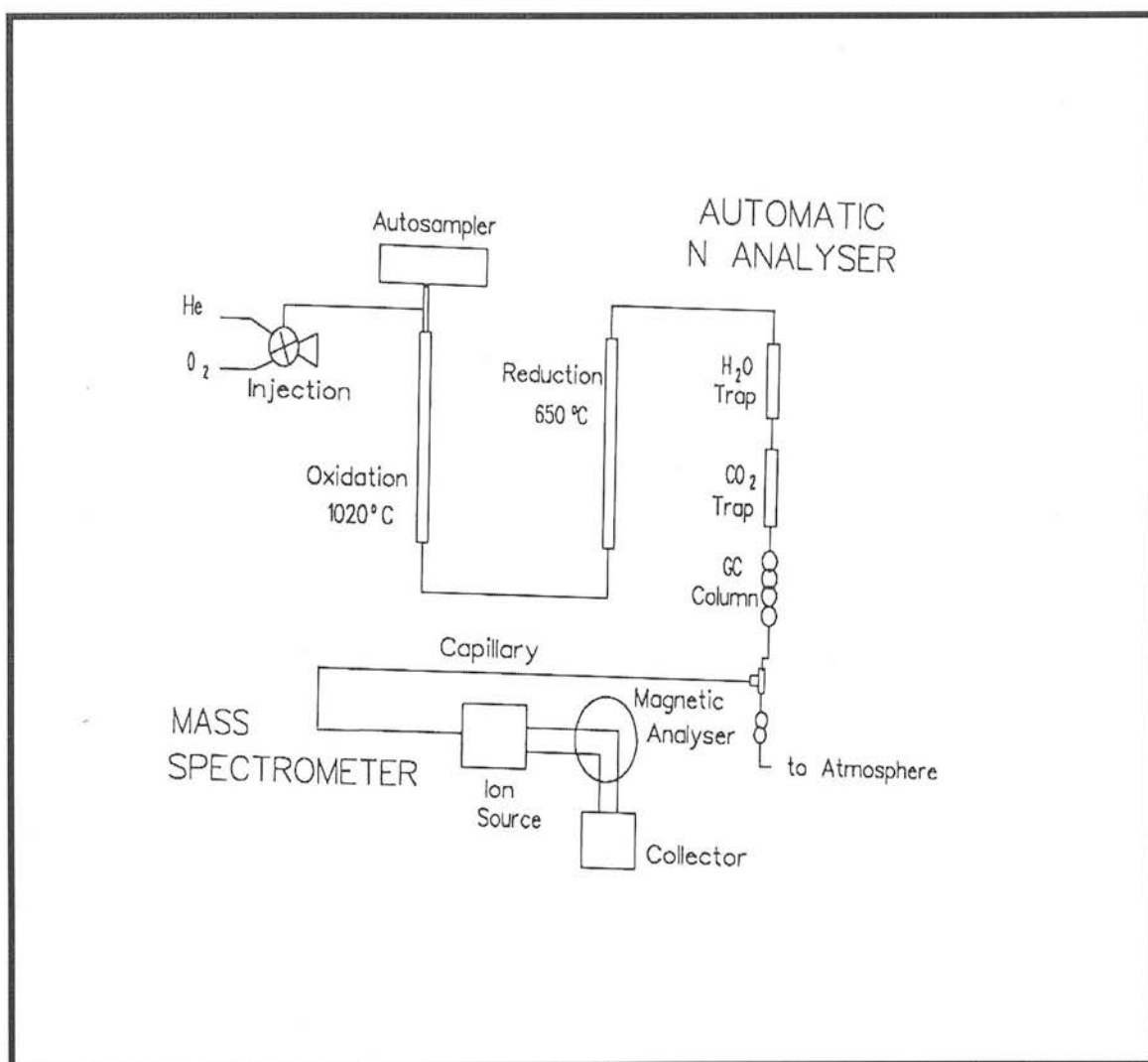


Figure 3.2 Schematic diagram of a mass spectrometer, linked to an automatic N analyser, as used in the determination of the ^{15}N enrichment of soil samples (Robinson and Smith, 1991).

4 SECONDARY WASTEWATER IRRIGATION AND SOIL PROPERTIES

4.1 Introduction

Large amounts of water are discharged from sewage plants in arid and semi-arid regions and in humid regions. Most of this water finds its way into streams. This water may be viewed as a wasted resource or as a pollution problem.

Irrigation is a standard practice in arid and semi-arid climates, especially in the Mediterranean area where about 70 to 90% of water resources are used for irrigation (Panoras and Ilias, 1999) because intensive cropping cannot depend on rainfall alone. Sorghum (*Sorghum bicolor* L.) and wheat (*Triticum aestivum* L.) production have been increased under irrigation conditions compared to their production under rainfed conditions (Proffit *et al.*, 1985). Since water is a limited resource in arid and semi-arid regions, the use of wastewater for irrigation in these regions has been of considerable interest, especially in the last two decades (Feigin *et al.*, 1991; Pescod, 1992). In Mexico for example, 82,900 hectares are already irrigated with wastewater (Strauss and Blumenthal, 1989). More than 70% of reclaimed municipal wastewater in California is used for irrigation (California State Water Resources Control Board, 1990). Reclaimed municipal wastewater plays an important role in Israel's agriculture where 72 % of this water is used for irrigation (Shelef and Azov, 1995). In the United States, municipal treatment plants throughout the country handle 19.8 billion gallons (20 million m³) of wastewater each day (Galegar, 1968) and about 1.65 Mm³ day⁻¹ is discharged from municipal wastewater treatment plants in Greece (Technical Chamber of Greece, 1993). Wastewater reuse represents a valuable water resource for agriculture practices and it could also substantially minimize environmental pollution problems.

The suitability of waters for irrigation is evaluated on the basis of their potential to create soil conditions hazardous to plant growth, and the health of crop consumers. Besides crop yield and crop quality, soil fertility, ground water properties and environmental quality are also affected by the quality of irrigation water. Wastewater quality is determined by its source and degree of treatment processes

(Thomas and Law, 1968). Treated wastewater usually does not contain settleable solids, although it usually does contain a certain amount of suspended organic and inorganic solids. Primary treatment of municipal wastewater may remove 25 to 40 % of the suspended solids. However, this can be increased up to 95% by using good secondary treatment processes (Thomas and Law, 1968). Organic compounds applied to the soil through wastewater irrigation decompose with time into CO₂, water and inorganic constituents. These inorganic constituents may be exchanged, adsorbed or precipitated, or might be taken up by plants and consequently be partially removed from the flowing solution.

Secondary municipal wastewater contains different concentrations of soluble and insoluble constituents that determine crop production through their immediate effect or by their gradual accumulation in soil. These constituents include salts, nutrients, trace elements and organic compounds. Moreover, secondary municipal wastewater usually contains appreciable amounts of sodium, which is an undesirable constituent of irrigation water. After it has been applied to the soil, wastewater mixes with the existing soil water and becomes a part of the soil system. As a result, it may alter the nature and rate of the physical, chemical and biochemical soil processes. The fate of materials added through wastewater irrigation to the soil system depends on their characteristics as well as on soil type, vegetation, climate and management factors.

The insoluble constituents such as suspended solids, particularly organic and inorganic precipitates, are quickly filtered from effluent irrigation water and accumulate in surface soil horizons. These suspended solids clog soil pores and may cause a reduction in soil hydraulic conductivity. On the other hand, soluble constituents may penetrate deeper into the soil profile. Constituents that have very high solubility may penetrate through the soil profile even before interacting with the soil system.

Many studies have evaluated the effect of treated municipal wastewater on irrigated soil properties (Bole *et al.*, 1981; Hayes *et al.*, 1990; Feigin *et al.*, 1991; Abo-Ghobar, 1993; Al-Jaloud *et al.*, 1993; Bajwa *et al.*, 1993; Panoras *et al.*, 2001a,

2001b). These studies have shown that wastewater can cause both beneficial and detrimental effects and that municipal wastewater generally has medium to high salinity, accompanied by high concentrations of sodium relative to the other cations. A high sodium level, which is quantified by the sodium adsorption ratio (SAR), may induce a sodicity problem through an increase in the exchangeable sodium percentage (ESP) in the soil. This sodicity hazard may result in a deterioration of soil structure through dispersion of clay particles, and consequently reduce soil porosity, hydraulic permeability and infiltration rate. Other effects include an increase in soil alkalinity and the concentration of both $\text{CO}_3^{2-} + \text{HCO}_3^-$ ions in the irrigation water, which may increase the sodicity hazard of the applied water by precipitation of calcium carbonate. The toxicity of specific ions and trace elements such as B and Cl⁻ that may have direct or indirect effects on plant, and nutritional imbalances, also might be increased (Feigin *et al.*, 1991).

Despite the occurrence of potential hazardous elements in wastewater, beneficial nutrients are also present. Secondary wastewater typically contains about 10-50 mg N l⁻¹ including 0-10 mg NO₃⁻-N l⁻¹ and 1-40 mg NH₄⁺-N l⁻¹ (Thomas and Law, 1977; Idelovitch, 1978; Asano *et al.*, 1985), and other essential plant macronutrients such as phosphorus, potassium, calcium and magnesium (see Table 2.1). Al-Jaloud *et al.* (1993) observed that the increase in green matter of maize and sorghum was attributable to the high level of nitrogen in the wastewater used for irrigation. However, a slight decrease in green matter was observed when using high salinity wastewater (2330 mg l⁻¹ TDS). These results indicate that nutrients in wastewater may be useful up to a certain water salinity level, but that any further increase in water salinity could neutralize the beneficial effects, and even cause a considerable yield reduction. Long-term wastewater irrigation (for 80 years) increased soil organic matter contents by 2.5 fold (Friedel *et al.*, 2000). However, wastewater irrigation decreased the soil infiltration rate because of an accumulation of suspended solids at the soil surface (Bajwa *et al.*, 1993).

The main objective of the current study was to evaluate the impact on soil physical and chemical properties by irrigating the soil with secondary municipal wastewater

(Q1) supplied by a furrow for four years, and comparing the effects with well water irrigation (Q3) at the Galicos River Wastewater Treatment Project in Greece.

4.2 Materials and methods

4.2.1 Field site

The soil sampling site is located at 40° 40' N latitude, 22° 48'E longitude in the northern part of Greece near the wastewater treatment plant of Thessaloniki city in a 0.25 ha area designed by the Galicos River Wastewater Treatment Project, established in 1996. The soil consists of sediments originating from the Galicos watershed. The clay type at the field site is kaolinite; the land topography is flat (0% slope). There is no artificial drainage system in the project area, but no water table was detected down to a depth of 3 m. The soils at the field site are characterized by low organic matter (an average of 1.7%) and classified at the great group level in the USDA system (Soil Taxonomy Soil Survey Staff, 1998) as Torrifluvents.

The cultivated crops included sugar beet (*Beta vulgaris* L.) in 1996 and 1997, cotton (*Gossypium hirsutum* L.) in 1998 and maize (*Zea mays* L., ssp. Mays) in 1999 and 2000. Management practices included synthetic soil fertilizer applications and chemical control of weeds and pests. Phosphorus and potassium fertilizers are provided annually through direct addition to the soil at rates ranging from 60 to 100 fertilizing units (fu) ha⁻¹ of 0-20-0 (N-P-K) to 400 - 500 fu ha⁻¹ of 0-0-50 (N-P-K) before sowing and during spring time respectively, where 1 fertilizing unit (fu) is equivalent to 1 kg of fertilizer material, while nitrogen fertilizer is applied at the rate of 60-160 fu ha⁻¹ of 21-0-0 (N-P-K) and 60 fu ha⁻¹ of 33.5-0-0 (N-P-K) during spring and summer time respectively. However, there is no soil management programme in the project to reduce soil salinity under wastewater irrigation. Irrigation management programmes were based on crop evapotranspiration estimated from data from a nearby meteorological station. The various irrigation treatments are shown in Figure 4.1.

The field site exhibits a semi-arid Mediterranean climate, with hot dry summers and warm rainy winters. The average monthly rainfall and temperature (Figure 4.2) were estimated from climatic data obtained from the Sindos climatological station located near the field site and the Axios Delta site (Chapter 7) (see map 3.1) for the period from January 1998 to October 2000. The mean annual temperature is in the range of 14-16 °C. The dry hot summer season spans from June to August and has maximum temperatures of between 29 and 41°C and minimum temperatures of 10-13 °C. The cold winter season spans from December to March and has maximum temperatures of 17-21°C and minimum temperatures between -2 and -7 °C. The mean annual precipitation is about 500 mm.

4.2.2 Irrigation water quality

The field experiment layout, involving three qualities of irrigation water is shown in Figure 4.1. Secondary wastewater from local sewage treated by stabilization ponds and sand filtration (Q1), biologically treated wastewater (Q2), and fresh water (well water) (Q3) were used. These water sources had been applied by drip (D) and furrow (F) irrigation for four years to plots, each with an area of 21 m².

Laboratory work was carried out on surface soil samples from selected plots irrigated with two sources of irrigation water: either secondary wastewater from local sewage treated by stabilization ponds and sand filtration (Q1), or fresh water (Q3) supplied by furrow for four years.

The chemical characteristics of wastewater (Q1) and well water (Q3) are shown in Table 4.1 (Panaras *et al.*, 1999). Well water was classified as C3-S1 (medium to high salinity and low sodicity hazard) using the United States Salinity Laboratory Staff classification of irrigation water (Richards, 1954). Wastewater was classified as C4-S2, as it has a very high salinity and slight to medium sodicity risk.

The suitability of wastewater for irrigation was evaluated. The theoretical pH value (pH_c) and adjusted-SAR (SAR_{adj}) of wastewater were calculated as described by Ayers (1977). The pH_c value of wastewater (6.69) was less than 8.4 (Table 4.1). A pH_c value lower than 8.4 indicates a certain tendency for CO₃²⁻ and HCO₃⁻ in

irrigation water to precipitate calcium in irrigated soil as CaCO_3 , with a corresponding increase in SAR of soil water (Ayers, 1977). The pHc values below 8.4 indicate a tendency to precipitate lime, while pHc values above 8.4 indicate a tendency to dissolve lime from the soil through which the water moves (Ayers, 1977). In addition, the calculated SAR_{adj} value of 27.42 (Table 4.1) suggested that some permeability problems may exist due to the effects of carbonate and bicarbonate ions on calcium availability. However, the residual sodium carbonate (RSC) value of $-3.65 \text{ mmol}_c \text{ l}^{-1}$ (Table 4.1) is less than $2.5 \text{ mmol}_c \text{ l}^{-1}$. This indicates that the quality of the municipal secondary wastewater from the city of Thessaloniki is regarded as safe to use for irrigation without any expected hazard of Na_2CO_3 formation (Eaton, 1950), otherwise, it is likely that the replacement of calcium and magnesium in the exchange soil sites by sodium causes progressive destruction of particle aggregates and decreases permeability. Moreover, the high salinity level of the wastewater (3.49 dS m^{-1}) (Table 4.1) could alleviate problems of high sodium levels under wastewater irrigation, because increasing salinity reduces the sodium potential to deflocculate soil particles (Oster and Schroer, 1979).

pHc, SAR_{adj} and RSC values for well water were not calculated because the concentrations of HCO_3^- ions were not obtained (Table 4.1).

Table 4.1 Chemical properties of wastewater and well water used to irrigate soil plots. Values in group (A) represent the average (n=11) (Panoras *et al.*, 1999), those in group (B) were calculated based on data in group (A). (- data not obtained, = data not calculated).

Parameter (A)	Irrigation water quality	
	Wastewater (Q1)	Well water (Q3)
COD (mg O ₂ l ⁻¹)	97	-
BOD ₅ (mg O ₂ l ⁻¹)	12.4	1.00
TSS ^a (mg l ⁻¹)	50	11
pH	7.97	7.70
EC (dS m ⁻¹)	3.49	0.96
TDS (mg l ⁻¹)	2113	614
Na ⁺ (mg l ⁻¹)	573	63.2
Ca ⁺⁺ (mg l ⁻¹)	100.5	-
Mg ⁺⁺ (mg l ⁻¹)	85	-
SAR (mmol _c l ⁻¹)	10.12	0.23
K ⁺ (mg l ⁻¹)	35.4	3
Cl ⁻ (mg l ⁻¹)	845	99.5
CO ₃ ⁻ (mg l ⁻¹)	0.0	0.0
HCO ₃ ⁻ (mg l ⁻¹)	516	-
NH ₄ ⁺ -N (mg l ⁻¹)	1.50	1.19
NO ₃ ⁻ -N (mg l ⁻¹)	22.7	1.3
(B)		
pH ^c	6.69	=
SAR _{adj} (mmol _c l ⁻¹)	27.42	=
RSC (mmol _c l ⁻¹)	-3.65	=

where

TSS^a = Total suspended solids

pHc^b = the theoretical pH value of wastewater; pHc was calculated by the following equation:

$$\text{pHc} = (\text{pK}_2' - \text{pKc}') + \text{p}(\text{Ca}^{++} + \text{Mg}^{++}) + \text{pAlk} \quad (\text{Equation 4.1})$$

(Ayers, 1977)

where

pK_2' is the negative logarithm of the second dissociation constant of H_2CO_3 .

pKc' is the negative logarithm of the solubility constant of CaCO_3 .

$\text{p}(\text{Ca}^{++} + \text{Mg}^{++})$ is the negative logarithm of the molar concentration of $\text{Ca}^{++} + \text{Mg}^{++}$.

pAlk is the negative logarithm of the equivalent concentration of treatable base (CO_3^{--} and HCO_3^-).

(All concentrations are in $\text{mmol}_e \text{ l}^{-1}$)

pHc was calculated by this equation using appropriate tables (Ayers, 1977) in which $(\text{pK}_2' - \text{pKc}')$ was obtained from tables by using the water analysis for $\text{Ca}^{++} + \text{Mg}^{++} + \text{Na}^+$; $\text{p}(\text{Ca}^{++} + \text{Mg}^{++})$ was obtained using the analysis for $\text{Ca}^{++} + \text{Mg}^{++}$; and pAlk was obtained from analysis for CO_3^{--} and HCO_3^- .

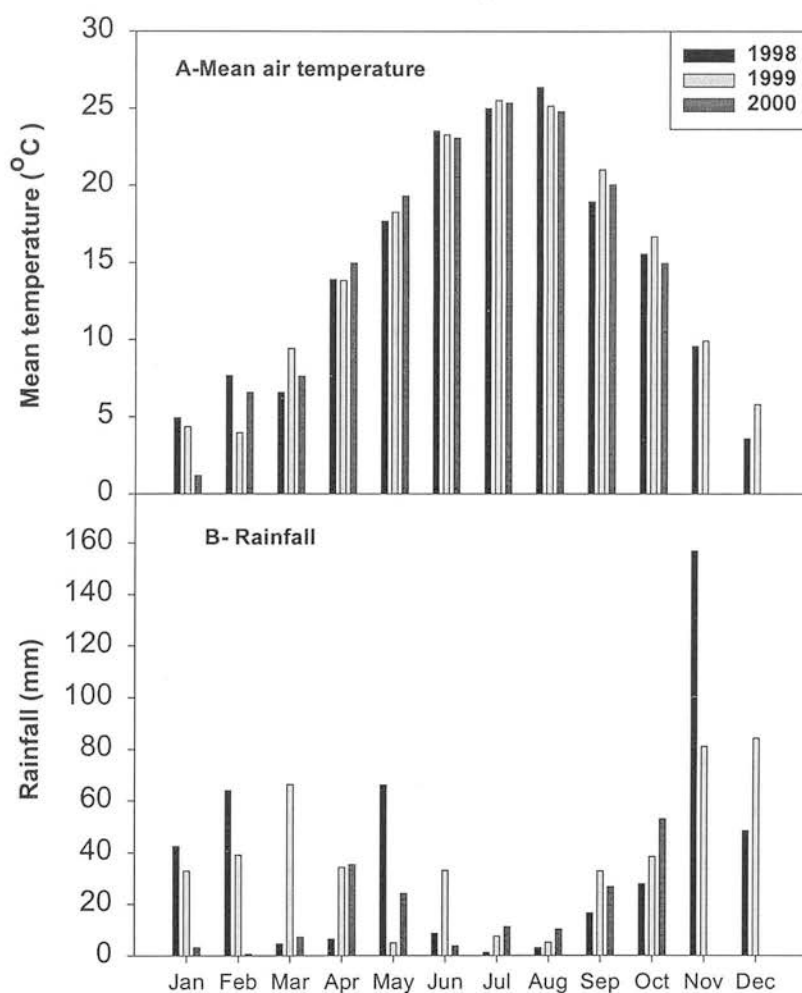


Figure 4.2 The mean monthly climatic data at the Galicos River site estimated from data obtained from Sindos Climatological Station (kindly provided by Panoras Athanasios, Galicos River Wastewater Treatment Project).

4.2.3 Soil sampling and analysis

Fresh, loose soil samples (0-5 cm depth), including surface residues were collected at the end of the irrigation season (August 2000) by staff from the Galicos River Wastewater Treatment Project from 6 plots irrigated with secondary municipal wastewater (Q1) and another 6 plots irrigated with fresh water (well water) (Q3). Soil samples from each selected plot were mixed to form a composite sample and stored at 4 °C for as short a time as possible prior to analysis. Three subsamples from each composite soil sample were analysed to evaluate the effect of secondary wastewater irrigation *versus* well water irrigation on physical and chemical soil properties. Three subsamples from each composite soil sample were taken and repacked in metal rings. The resulting repacked soil cores were saturated using tap water to prevent dispersion of soil aggregates, placed on a tension table and subjected to different water tensions. The relationship between soil water suction and moisture content was plotted in the graph and represented a soil moisture release curve. Another three portions were also taken from each fresh, loose composite soil sample, air-dried and repacked in leaching columns to measure saturated hydraulic conductivity. The methods used for soil analysis are listed in Table 4.2. For details of general methodology see Chapter 3.

4.2.4 Data analysis

Data was prepared and tabulated in Excel. Means of observations and standard deviations of means were calculated. The significance of the difference between soil properties after irrigation with wastewater and well water were tested using a Two-Sample t-test at a confidence interval for differences in means of 95% (Ryan and Joiner, 2001; Mead *et al.*, 2003). All statistical analyses were carried out using MiniTab 12.

Table 4.2 Methods, instruments and references used in the soil physical and chemical analysis.

Parameter	Method	Instrument	Reference	Page
Moisture content	Gravimetric with oven drying method		Gardner, 1986	41
pH	1:2.5 soil:water ratio suspension	Glass electrode-pH meter	Black <i>et al.</i> , 1965	43
EC _c (dS m ⁻¹)	saturated soil paste extraction	Wheatstone bridge	Richards, 1954	44
Soluble cations (mg l ⁻¹) (Na ⁺ , K ⁺ , Ca ⁺⁺ , Mg ⁺⁺)	using ECe-extract	(ICPAAS TM 61E Spectrometer)		45
Soluble anions (mg l ⁻¹) Cl ⁻	using ECe -extract	Reference and chloride electrode with pH meter.	Adriano and Doner, 1982	46
CO ₃ ²⁻ and HCO ₃ ⁻ (mg l ⁻¹)	using ECe- extract	Titration equipment	Hesse, 1971	46
Water-soluble organic carbon (µg C g ⁻¹ dry soil)	using ECe- extract	Dohrmann DC-80 TOC Analyser		48
Exchangeable cations (Na ⁺ , K ⁺ , Ca ⁺⁺ , Mg ⁺⁺) (mmol _c 100 g ⁻¹ soil)	Ammonium acetate extraction	(ICPAAS TM 61E Spectrometer)	Bower <i>et al.</i> , 1952	49
Cation exchange capacity (mmol _c 100 g ⁻¹ soil)	Sodium acetate extraction	ICPAAS TM 61E Spectrometer)	Bower <i>et al.</i> , 1952	51
Particle size distribution	Hydrometer method	Standard hydrometer ASTM no. 152 H	Black <i>et al.</i> , 1965	52
Soil organic carbon (mg C g ⁻¹ dry soil)	Dichromate method	Titration equipment	Rowell, 1994	54
CaCO ₃ %	Titration with NaOH	Titration equipment	Rowell, 1994	54
Mineral nitrogen NH ₄ ⁺ -N and NO ₃ ⁻ -N (µg. N g ⁻¹ dry soil)	1 M KCl ⁻ - extraction	Chemlab Instruments Autoanalyser	Bremner, 1965	56

Table 4.2 continued

Parameter	Method	Instrument	Reference	Page
Total nitrogen -%	Kjeldahl method	Micro-Kjeldahl	(Büchi Nitrogen information - leaflet No.1)	59
Soil strength (kg cm ⁻²)	Drop cone penetrometry	Drop cone penetrometer	Bradford, 1986	60
Infiltration rate (cm h ⁻¹)	Single ring infiltrometry	Soil cores	Bouwer, 1986	61
Water retention	Tension table	Tension table	Topp <i>et al.</i> , 1993	62
Saturated hydraulic conductivity (cm h ⁻¹)	Constant head method	Leaching soil columns	Klute and Dirksen, 1986	66

4.3 Results

There was a very significantly higher clay content ($T = 6.67$ $p < 0.001$ $DF = 34$), and lower sand content ($T = -4.24$ $p < 0.001$ $DF = 34$) in the wastewater-irrigated soils. The maximum soil moisture percent observed in soil samples irrigated with wastewater was 12.4%. However, only 8.4% soil moisture was detected in soil samples irrigated with well water (Table 4.3). The difference in soil moisture content between the two treatments was highly significant ($T = 8.74$ $p < 0.001$ $DF = 34$) and a strong positive correlation was found between soil moisture content and clay percent in soil irrigated with wastewater ($r = 0.81$).

Soil irrigated with wastewater does seem to have had higher water content over the drainage period compared with soil irrigated with well water (Figure 4.3). Water content at saturation was greater in well water-irrigated soils than wastewater-irrigated soils but the reverse was true after soil samples were allowed to drain for approximately one hour.

Soils of both irrigation treatments seem to have the same water content over a range of water tensions from 0 to -18 kPa (Figure 4.4).

Generally, the saturated hydraulic conductivity (K_{sat}) of irrigated soils was low, averaging 0.98 ± 0.18 and 1.08 ± 0.09 cm h⁻¹ for wastewater and well water-irrigated soil respectively (Table 4.3, Figure 4.5). Statistically, the difference in saturated hydraulic conductivity between irrigated soils was not significant.

Wastewater irrigation resulted in a highly significant increase in electrical conductivity (EC) of irrigated soil ($T = 23.26$ $p < 0.001$ $DF = 34$), compared with that of the soil irrigated with well water (Table 4.3). These results were consistent with salt loading for the different irrigation sources (Table 4.1).

Wastewater-irrigated soil (Table 4.3) had a highly significant increase in soluble Na⁺ ($T = 29.87$ $p < 0.001$ $DF = 34$) and soluble Mg⁺⁺ ($T = 5.49$ $p < 0.001$ $DF = 34$) compared with well water -irrigated soils. However, it had a highly significant decrease in soluble Ca⁺⁺ ($T = -11.56$ $p < 0.001$ $DF = 34$). There was no significant difference in concentration of soluble K between the two irrigation treatments.

Chloride concentration in soil irrigated with wastewater very significantly increased ($T = 19.44$ $p < 0.001$ $DF = 34$) compared to those irrigated with well water. These results were consistent with chloride concentrations for the different irrigation sources (Table 4.1). Moreover, chloride concentrations recovered in both irrigated soils (Table 4.3) were lower than the amounts applied in the irrigation water sources (Table 4.1).

A strong positive correlation was found between soluble Na⁺, Ca⁺⁺, Mg⁺⁺ cations and Cl⁻ concentration and electric conductivity in saturated paste extracts of irrigated soils. In wastewater-irrigated soil extracts $r = 0.96, 0.81, 0.84$ and 0.97 for soluble cations and Cl⁻ ion concentration respectively, and in well water-irrigated soil extracts $r = 0.79, 0.98, 0.92$ and 0.95 , respectively.

Wastewater irrigation resulted in a highly significant increase in HCO₃⁻ concentrations ($T = 38.23$ $p < 0.001$ $DF = 34$) compared with those after well water

irrigation, while CO_3^{--} concentrations under both irrigation treatments were not detectable.

Comparison of wastewater and well water irrigation effects using the Two-Sample t-test showed a highly significant increase in CaCO_3 content ($T = 16.23$ $p < 0.001$ $DF = 34$), water-soluble organic carbon (WSOC) concentration ($T = 9.90$ $p < 0.001$ $DF = 34$), pH ($T = 36.39$ $p < 0.001$ $DF = 34$) and NO_3^- -N ($T = 5.63$ $p < 0.001$ $DF = 34$) when soils were irrigated with wastewater. However, there was no significant difference in NH_4^+ -N, or in organic carbon content between both irrigated soils.

A highly significant increase in concentrations of Na^+ ($T = 9.17$ $p < 0.001$ $DF = 34$), Mg^{++} ($T = 5.51$ $p < 0.001$ $DF = 34$) and exchangeable K^+ ion ($T = 5.01$ $p < 0.001$ $DF = 34$) was detected on the cation exchange sites of wastewater-irrigated soils (Table 4.3). However, the concentrations of exchangeable Ca^{++} were decreased very significantly ($T = -7.14$ $p < 0.001$ $DF = 34$) compared to soils irrigated with well water. No significant difference was detectable in the total quantity of negative charges (cation exchange capacity) between the two treatments.

Irrigation with wastewater of salinity 3.49 dS m^{-1} , TDS 2113 mg l^{-1} and SAR_{iw} 10.12 (Table 4.1) resulted in a highly significant increase in exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) of irrigated soil compared with well water irrigation ($T = 7.78$ $p < 0.001$ $DF = 34$), ($T = 52.21$ $p < 0.001$ $DF = 34$). As a result, more clay dispersion occurred and was visually detectable on the soil surface layer of wastewater-irrigated soil (Plate 4.1), compared with the surface layer of soils irrigated with well water ($\text{SAR}_{\text{iw}} = 0.23$) (Plate 4.2). In addition, wastewater irrigation led to a decrease in soil infiltration rate (IR) (Figure 4.6).

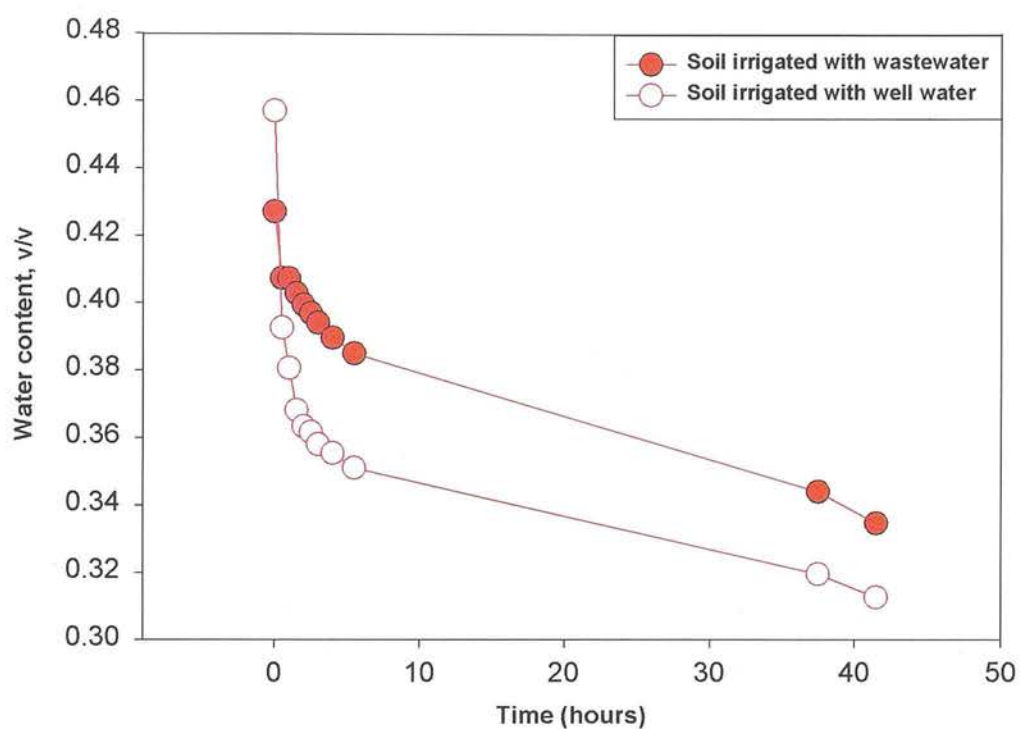


Figure 4.3 Change in soil water content with time after infiltration ceased in soils irrigated either with wastewater or well water. One soil core was used for each treatment.

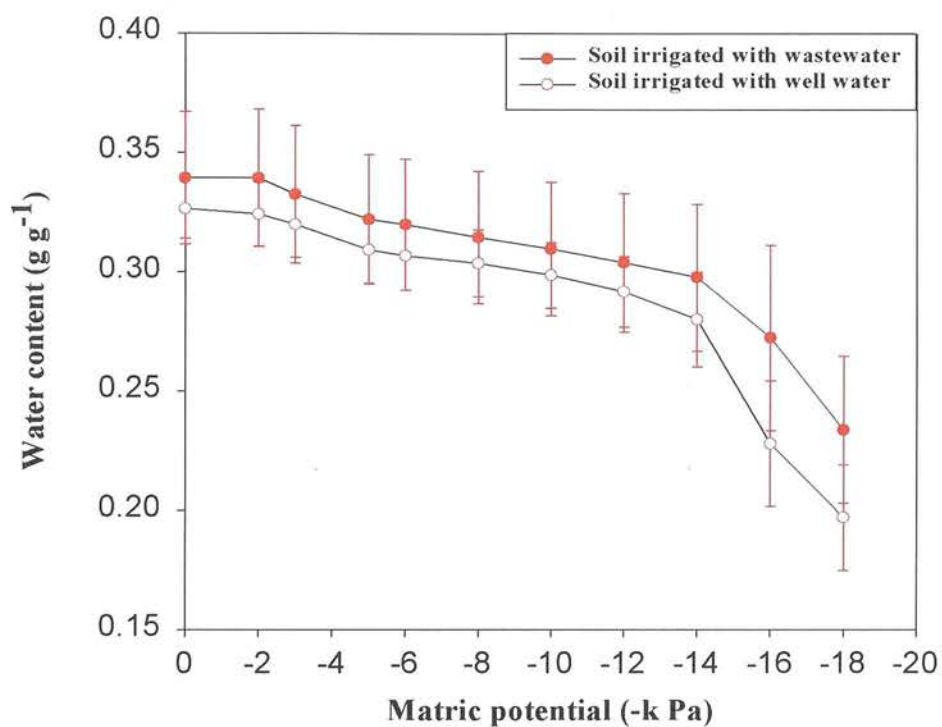


Figure 4.4 Water release characteristics of soil irrigated with wastewater and soil irrigated with well water over a range of water potential from 0 to -18 kPa. Error bars represent standard deviation of the means of six plots.

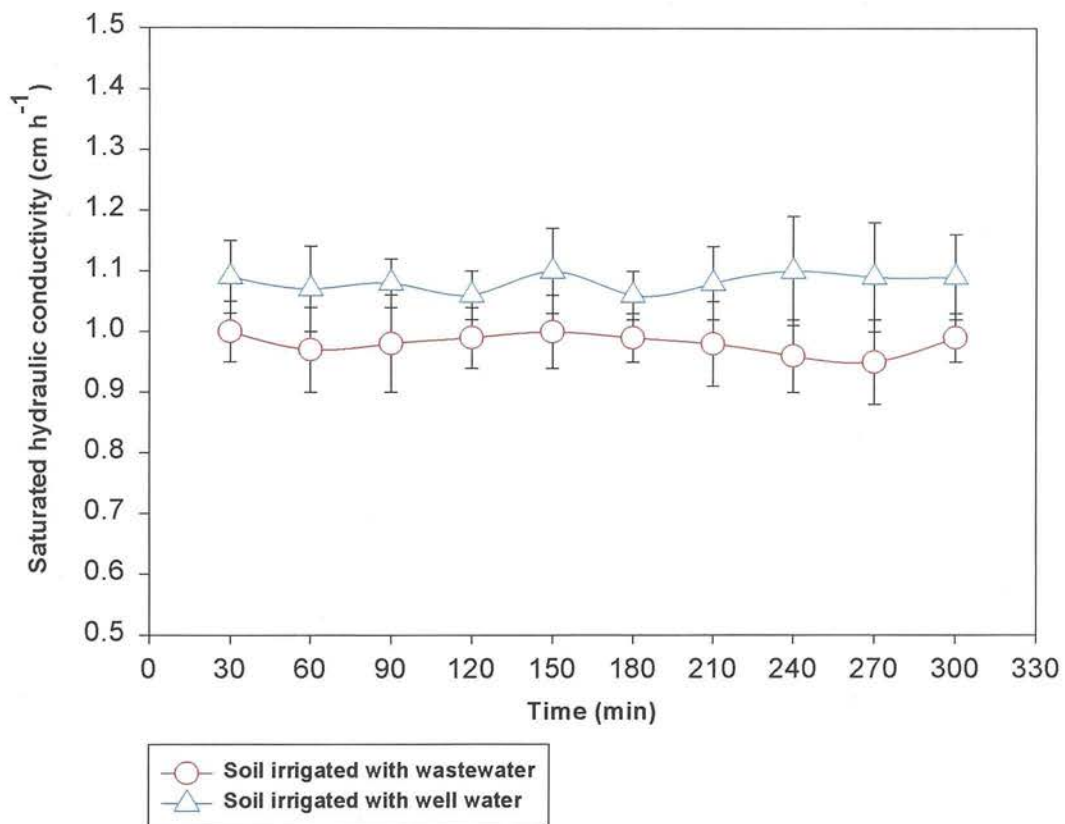


Figure 4.5 Effect of irrigation water quality (wastewater versus well water) on saturated hydraulic conductivity of irrigated soil. Error bars represent standard deviation of the means of six plots.

Table 4.3 Chemical and some physical properties of irrigated soil. Values represent means \pm s.d (n=18). Means followed by the same letter for each parameter under both irrigation treatments are not significantly different at $P<0.05$.

Parameter	Soil irrigated with wastewater			Soil irrigated with well water		
	Min	Max	Mean \pm s.d	Min	Max	Mean \pm s.d
Moisture content (%)	8.00	12.4	10.6 \pm 1.47	3.9	8.4	6.1 \pm 1.65
pH	8.6	8.8	8.7 \pm 0.03	8.3	8.4	8.3 \pm 0.03
EC (dS m ⁻¹)	2.0	3.0	2.5 \pm 0.35	0.5	0.6	0.5 \pm 0.05
Soluble cations (mg l ⁻¹)						
Na ⁺	428	613	506 \pm 64.4	39.5	60.3	49.3 \pm 7.85
K ⁺	4.80	15.5	7.9 \pm 3.76 ^a	3.3	8.0	6.0 \pm 1.35 ^a
Ca ⁺⁺	36.2	57.2	42.5 \pm 7.07	61.4	77.4	67.1 \pm 5.58
Mg ⁺⁺	35.7	50.9	42.2 \pm 5.20	30.3	39.9	34.2 \pm 3.26
Soluble anions (mg l ⁻¹)						
Cl ⁻	51.1	89.1	70.8 \pm 14.8	2.0	4.0	2.7 \pm 0.65
HCO ₃ ⁻	12	13	12.2 \pm 0.38	7.5	8.4	7.8 \pm 0.31
Exchangeable cations (mmol _c 100g ⁻¹ soil)						
Na ⁺	0.5	1.6	1.2 \pm 0.51	0.08	0.10	0.09 \pm 0.009
K ⁺	0.1	0.3	0.2 \pm 0.04	0.09	0.20	0.1 \pm 0.04
Ca ⁺⁺	2.4	3.3	2.9 \pm 0.30	3.2	5.0	3.9 \pm 0.54
Mg ⁺⁺	3.3	6.8	5.1 \pm 1.10	2.9	4.1	3.5 \pm 0.46
CEC (mmol _c 100 g ⁻¹ soil)	8.0	13.5	10.0 \pm 1.11 ^b	7.5	11	8.9 \pm 0.95 ^b
ESP (%)	5.9	23.5	12.0 \pm 1.6	1.0	1.5	1.0 \pm 0.19
SAR (mmol _c l ⁻¹)	11.9	14.1	13.1 \pm 0.95	1.0	1.4	1.2 \pm 0.14
Soil organic carbon (mg C g ⁻¹ dry soil)	9.6	11	10.1 \pm 0.45 ^c	9.0	12	10 \pm 1.05 ^c
water- soluble organic carbon (µg C g ⁻¹ dry soil)	41.8	54.1	47.6 \pm 4.88	31.8	39.9	34.6 \pm 2.67
NH ₄ ⁺ -N (µg g ⁻¹ dry soil)	0.05	0.90	0.3 \pm 0.02 ^d	0.05	0.80	0.1 \pm 0.02 ^d
NO ₃ ⁻ -N (µg g ⁻¹ dry soil)	1.4	2.7	1.9 \pm 0.31	0.60	1.30	1.1 \pm 0.26
CaCO ₃ (%)	3.0	4.2	3.8 \pm 0.41	2.0	2.4	2.1 \pm 0.17
Sand (%)	17.9	46.2	26.9 \pm 10.3	28.1	43.5	38.4 \pm 5.08
Silt (%)	37.8	58.4	51.1 \pm 7.08 ^e	44	56	46.0 \pm 4.27 ^e
Clay (%)	15.9	26.1	21.9 \pm 3.94	13.4	15.9	15.5 \pm 0.97
Soil texture	Clay loam			Sandy silt loam		
Hydraulic conductivity (cm h ⁻¹)	0.6	1.3	0.98 \pm 0.18 ^f	1.0	1.2	1.08 \pm 0.09 ^f



Plate 4.1 Clay dispersion at the surface layer of soil irrigated with wastewater ($\text{SAR}_{\text{iw}} = 10.12 \text{ mmol}_c \text{ l}^{-1}$).



Plate 4.2 Surface layer of soil irrigated with well water ($\text{SAR}_{\text{iw}} = 0.23 \text{ mmol}_c \text{ l}^{-1}$).

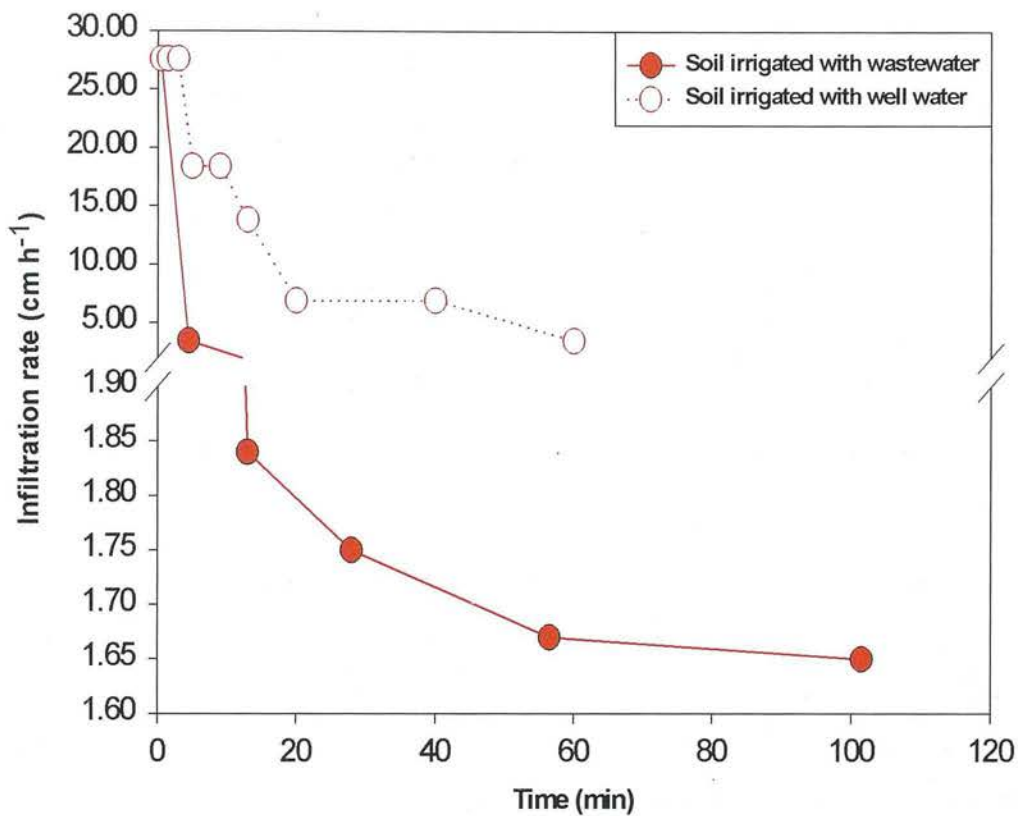


Figure 4.6 Effect of irrigation water quality (wastewater versus well water) on soil infiltration rate. Initial moisture contents were $\theta = 0.138$ and 0.079 respectively. Infiltration rate values were determined using single cores.

4.4 Discussion

There were no differences in water contents of irrigated soils over the range of matric potential from 0 to -18 kPa. These results suggest that there might be a similarity in pore size distribution in both irrigated soils.

Soil irrigated with wastewater does seem to have had higher moisture content over the drainage period compared with soil irrigated with well water. This is probably due to the differences in their soil textures. This was consistent with the highly significant higher clay content of soil irrigated with wastewater, which increased the water holding capacity.

According to the range of conductivity values in soils reported by Klute and Dirksen (1986), the saturated hydraulic conductivity (K_{sat}) of the irrigated soils under study is low. This might be due to the low organic matter content of these soils. No significant difference was observed in the saturated hydraulic conductivity (K_{sat}) of irrigated soils. This has strong implications, since hydraulic conductivity (HC) is one of the soil properties which most determines the behaviour of the soil water flow system, has effects directly or indirectly on plant growth, and also provides a measure of a soil ability to transmit water and move salts away from the root zone. These results suggest that wastewater irrigation over four years had no adverse effect on the soil hydraulic conductivity, and are in agreement with those obtained in other studies (Balks *et al.*, 1998; Panoras *et al.*, 2001a, 2001b). Panoras *et al.* (2001a, 2001b) observed no soil deterioration relevant to the reduction in HC at the same field site under study using the same quality of wastewater irrigation to grow cotton and corn. Moreover, the magnitude of the measured decrease in K_{sat} in wastewater-irrigated soil was not sufficient to limit water flow through the soil profile, and the variety Dias (NAGERF-Cereal Institute) of corn and the variety 83849-51 (NAGERF-Cotton Institute) of cotton were grown successfully. In addition, a good productivity was achieved (Panoras *et al.*, 2001a, 2001b).

The pH of soil irrigated with wastewater was very significantly higher (pH = 8.7 for wastewater vs pH = 8.3 for well water-irrigated soil). This could be attributed to the displacement of H^+ ions from the soil surface, caused by the addition of basic

cations and anions in the wastewater irrigation source. These results disagree with those reported in other studies estimating the effects of wastewater reuse on the pH of irrigated soil, in which the pH of soil irrigated with wastewater was significantly decreased (Hayes *et al.*, 1990). This discrepancy could be due to the nitrification effect following addition of nitrogen through the irrigation source in Hayes' study.

There was a highly significant increase in soil salinity under wastewater irrigation compared with well water irrigation. This indicates that soil salinity mirrors the salinity levels of irrigation water. These results are in agreement with other authors who reported significant increases in soil salinity with a corresponding increase in wastewater salinity (Hayes *et al.*, 1990; Al-Jaloud *et al.*, 1993; Falkiner and Smith, 1997).

Although the statistical analysis showed a highly significant increase in electrical conductivity (EC) of wastewater-irrigated soil compared with well water-irrigated soil, the electrical conductivity of saturated soil extracts of both of the irrigated soils under study averaged 2.5 and 0.56 dS m⁻¹ for wastewater and well water-irrigated soil, respectively. These results suggest that after 4 years of irrigation with highly saline wastewater (3.49 dS m⁻¹) there was no high salt accumulation in the irrigated soil. This might be attributed to the leaching processes by rainfall in autumn, winter, spring and during the time of sampling. These results indicate that drainage efficiency in the project, and soil physical and chemical properties such as hydraulic conductivity and possibly mineralogical composition helped to leach soluble salts accumulated during the dry season (McNeal and Coleman, 1966; Frenkel *et al.*, 1978). Accordingly, Panoras *et al.* (2001a, 2001b) reported that at the same study site where they were growing cotton and corn, the winter rainfall reduced the salinity hazard significantly under wastewater irrigation. The reduction in electrical conductivity of wastewater-irrigated soil, in spite of the salt added through irrigation over four years in our study, is encouraging for the continuous investment in wastewater for irrigation. Moreover, the average EC value of wastewater was not higher than 3.46 dS m⁻¹. This value is acceptable according to U.S salinity laboratory classification for irrigation water (Richards, 1954), especially for sugar beet, cotton and corn crops. Elsewhere, wastewater with salinity ranging from 1.5 to

4.6 dS m⁻¹ and SAR_{adj} ranging from 4.96 to 16.1 was used beneficially for growing maize (*Zea mays* L., a US cv. Atlantic Yellow Hybrid Sheet Corn) and forage sorghum (*Sorghum vulgare* Pers., a Sudan cv. Beef Builder) (Al-Jaloud *et al.*, 1993).

A strong positive correlation was found between soluble Na⁺, Ca⁺⁺, Mg⁺⁺ cations and Cl⁻ concentration and electric conductivity in saturated paste extracts of irrigated soils. Thus, we can say that the most dominant salt in soil irrigated with wastewater is sodium chloride and, to a lesser degree, calcium and magnesium chloride salts. However, in well water-irrigated soil, the most dominant salts are calcium and magnesium chloride salts and to a lesser degree, sodium chloride salt.

Wastewater irrigation produced soil with soluble sodium levels that were very significantly higher than those in well water-irrigated soil. This might be due to either the higher Na⁺ concentration in wastewater or the effect of high bicarbonate ion concentration in wastewater (Table 4.1) on precipitation of calcium (Oster and Schroer, 1979). The latter hypothesis is supported by the highly significant increase in CaCO₃ content followed by a highly significant decrease in soluble calcium under wastewater irrigation. In addition, the pH_c value for wastewater (Table 4.1) also indicated that CaCO₃ would readily precipitate under wastewater irrigation. These results are in agreement with other studies (Oster and Schroer, 1979; Bole *et al.*, 1981).

Soluble magnesium was very significantly increased under wastewater irrigation, while soluble calcium showed a highly significant decrease. This might be attributed to the effect of higher HCO₃⁻ concentrations in wastewater on the precipitation of calcium in the soil. Although statistical analysis showed a highly significant decrease in soluble calcium under wastewater irrigation, chemical analysis data (Table 4.3) showed that an appreciable amount of soluble calcium still remained in soil solution (saturated soil paste extract). This is a good property, opposing the dominance of the soluble sodium cation in soil solution and on soil exchange sites, which can lead to deterioration in soil physical properties.

The CO_3^{--} ion concentrations in saturated paste extracts of irrigated soils was not detectable. This might explain the alleviation of the adverse effects of high soluble sodium concentrations under wastewater irrigation. In the presence of high CO_3^{--} concentrations in soil water, soluble sodium in the soil solution reacts with this anion to form sodium carbonate. As a consequence, soils become strongly alkaline, soil particles disperse, and soil becomes unfavourable for the entry and movement of water. The result is a soil unfavourable for tillage. However, wastewater irrigation caused a highly significant increase in HCO_3^- ion concentrations in soil water compared to well water irrigation, which could reflect a high HCO_3^- ion concentration in wastewater.

Wastewater irrigation resulted in a highly significant increase in Cl^- concentration compared with well water irrigation. The Cl^- concentrations measured in both irrigated soils were lower than those present in the irrigation waters. This could be attributed to the leaching processes for salts that might be accumulated during the summer season by the action of rainfall during the rainy season. These results prove that the internal drainage of the irrigated soils in the area under study is suitable for leaching of salts down the soil profile through the percolation of the irrigation water.

Wastewater irrigation resulted in a highly significant increase in soil NO_3^- -N. This was attributable to the high NO_3^- -N concentration in the irrigation source compared to well water. These results are in agreement with another study reporting a significant increase in soil NO_3^- -N concentration after 16 months of secondary municipal wastewater irrigation, due to the high total N concentration in the wastewater source (Hayes *et al.*, 1990). Although statistical analysis in our study showed a significant increase in NO_3^- -N concentration under wastewater irrigation *versus* well water irrigation, the NO_3^- -N concentration in wastewater treated soils were lower than the NO_3^- -N concentration in the irrigation source. This is likely to be due to crop uptake of part of the NO_3^- -N.

The cation exchange capacity (CEC) of the irrigated soils was relatively low (averaging $10 \pm 1.11 \text{ mmol}_c \text{ } 100\text{g}^{-1}$ soil for wastewater-irrigated soils *versus* $8.9 \pm 0.95 \text{ mmol}_c \text{ } 100 \text{ g}^{-1}$ soil for well water-irrigated soil), and could be attributed to

the low organic matter and clay content of the irrigated soils. Although statistical analysis showed a significantly higher clay percentage content in wastewater-irrigated soil, the average percent of clay content in these soils was not higher than 21.9 %. These results suggest that this clay percentage content is not sufficient to increase the soil cation exchange capacity substantially.

Exchangeable calcium accounted for 43.8 % of the exchangeable cations on the exchange sites of the well water-irrigated soil. The corresponding value for the wastewater-irrigated soil was 29 %. The decrease in exchangeable calcium under wastewater irrigation was highly significant. This might be attributed to the effect of HCO_3^- ion concentrations on calcium precipitation as CaCO_3 , this anion being higher in wastewater. The solubility factor for CaCO_3 is 0.014 g l^{-1} whereas the solubility factor for MgCO_3 is higher (1.76 g l^{-1}). The effect of HCO_3^- ion concentrations in wastewater is to precipitate calcium as CaCO_3 but not to precipitate magnesium as MgCO_3 . The increase in Mg^{++} cation concentrations on soil exchange sites (Table 4.3) indicated that this effect had occurred.

The exchangeable sodium percent (ESP) on the soil exchange sites of wastewater-irrigated soil was increased significantly (by an average of 12.0 %) and resulted in clay dispersion in the surface layer of the soil. These observations are in agreement with results from another study (Balks *et al.*, 1998). As a result, the infiltration rate of the soil decreased sharply with time. However, it was only slightly decreased in soil irrigated with well water. This sharp decrease in IR of soil irrigated with wastewater might be due to the pore blockage because of surface clay dispersion (plate 4.1), compared with the surface layer of the soil irrigated with well water (plate 4.2). These results are in agreement with other studies investigating the influence of treated wastewater on soil infiltration rate (Abo-Ghobar, 1993; Bajwa *et al.*, 1993). However Panoras *et al.* (2001a, 2001b) who worked in the same field site under study reported that the reduction in infiltration rate of the wastewater-irrigated soil using the same quality of wastewater used in our study, was not sufficient to prevent the use of this wastewater to grow cotton and corn crops. In addition, no significant differences were observed in the yield of seed cotton of variety 83849-51 (NAGERF. Cotton Institute) and corn of Dias (NAGERF-Cereal

Institute) using the same wastewater and well water quality supplied by a furrow over four years (Panoras *et al.*, 2001a, 2001b).

The highly significant increase in ESP under wastewater irrigation might be attributed to the high sodium content relative to the calcium and magnesium in soil solution. Thus, the absorbed Ca^{++} and Mg^{++} of the soil were replaced by Na^+ . Experiments already conducted on the effect of total electrolyte concentration and sodium adsorption ratio of percolating solution on soil sodicity can be seen in Appendix 1. Many workers elsewhere have reported that soil sodicity has been shown to increase as the SAR of irrigation water increases (Paliwal and Grandhi, 1976; Al-Jaloud *et al.*, 1993). Although statistical analysis has shown a highly significant increase in ESP of soils irrigated with wastewater compared to soils irrigated with well water, the average ESP value of wastewater-irrigated soil was lower than the threshold value of 15 %, above which soil structure could be destroyed (Richard, 1954).

Exchangeable potassium accounted for a low percentage of the cation exchange capacity of the irrigated soils. Although statistical analysis has shown a significant increase in exchangeable potassium under wastewater irrigation, the concentration of potassium on exchange sites of wastewater-irrigated soil averaged 0.22 mmol_c 100 g⁻¹ soil, which corresponds to 2.2 % of cation exchange capacity. This low percentage might be due to a low concentration of soluble potassium in soil water. Another possibility is that a deficiency of minerals containing this cation occurred in our irrigated soils.

A non significant difference in soil organic carbon was observed for these two irrigation treatments (mean 10.1 mg C g⁻¹ dry soil for wastewater-irrigated soils *versus* mean 10 mg C g⁻¹ dry soil for well water-irrigated soils). These results suggest that some microbial mineralisation of organic carbon under wastewater irrigation occurred, possibly being favoured by the high temperature experienced by the field site, especially during the summer season. Friedel *et al.* (2000) reported that the total organic carbon contents (TOC) at 0-15 cm soil depth increased 2.5 fold after 80 years of irrigation with municipal wastewater. The differences from our

results were possibly due to the large quantity of wastewater that Friedel *et al.* (2000). used in their study, which therefore caused a large quantity of organic matter to be added. This was because of the high irrigation water requirement for alfalfa and for vertisols to reach field capacity. Another possibility is that the increase in total organic carbon contents in Friedel's study might have been due to the long-term irrigation used, whereas the period of our irrigation was much shorter. Alternatively it could be that the TOC % in the wastewater used in our study was low.

4.5 Overview

Irrigation of sugar beet, cotton and corn crops over four years with secondary wastewater treated by stabilization ponds and sand filtration, and with a very high salinity and slight to medium sodicity risk (C4-S2), resulted in both beneficial and some detrimental changes in soil properties. Irrigation with this wastewater quality increased soil salinity and soil sodicity and led to the production of sodium saline soils, as classified by Eaton (1950). In addition, wastewater resulted in a very highly significant increase in NO_3^- -N and water-soluble organic carbon (WSOC) concentration of irrigated soil.

The theoretical pH value (pH_c) of wastewater showed that CaCO_3 would readily precipitate under this wastewater irrigation. In addition, the adjusted-SAR value (SAR_{adj}) of wastewater suggested that some problems with permeability may exist due to the effects of carbonate and bicarbonate ions on calcium availability. However, winter rainfall had a significant effect in leaching any accumulated salts. Moreover, irrigation with this high salinity wastewater with no residual sodium carbonate should alleviate any problems caused by high sodium levels. The increase in irrigation water salinity reduces the potential of Na^+ to deflocculate soil particles (Oster and Schroer, 1979).

5 Denitrification and irrigation water quality

5.1 Introduction

Where high NO_3^- levels in soil and /or water present a pollution hazard, topsoil denitrification is considered a desirable process to remove NO_3^- that may otherwise be leached to ground and surface waters (Aulakh *et al.*, 1992; Bernet *et al.*, 1995). However, the gaseous N products of NO_3^- reduction in topsoil are likely to pose an environmental problem, unless reduction to N_2 is complete. N_2O is a greenhouse gas, contributing to global warming, and also causes depletion of the ozone layer (Groffman, 1995).

Irrigation with secondary wastewater not only provides water to the land, but also results in the addition of organic matter and a considerable amount of nitrogen to the soil (see Table 2.1). Biological Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD) are commonly used for evaluating the concentration of organic matter in sewage effluent (Feigin *et al.*, 1991).

As soon as the effluent-N reaches the soil, it becomes part of the soil N cycle. Thus, irrigation with wastewater affects N uptake by the crop, the level of available N in the soil, gaseous N losses and N leaching below the rooting depth. Since the amount of N in sewage effluents is high, the C/N ratio for the organic components is low. Typical organic C / organic N ratios of sewage effluents are around 5 or lower and the organic C / total N ratio is much lower (Feign *et al.*, 1991). These organic materials are easily decomposable and result in addition of available N to the soil. The application of sewage effluent of a high BOD_5 value reduces the oxygen level in the soil air, and consequently enhances denitrification (Feign *et al.*, 1991). High sodium content in wastewater has the ability to disperse soil, resulting in reduced hydraulic conductivity of the soil profile and infiltration rate of water and also leads to poor aeration and nitrogen losses through denitrification. Both the frequency and the amount of wastewater applied affect denitrification rates (Lance and Whisler, 1972), as do microbial respiration, soil moisture status and soil carbon and nitrogen dynamics (Rolston *et al.*, 1982; Sexstone *et al.*, 1985).

Studies that have examined the spatial variation of denitrification rates in the field reported that the water content in the surface soil was the main factor affecting variability in denitrification rates (Folorunso and Rolston, 1984; Burton and Beauchamp, 1985). Bremner and Shaw (1958a) observed a strong positive correlation between soil water content and denitrification rate. Data from experiments with depth probes suggested that the top 5 cm of the soil is the major zone of denitrifying activity in a clay loam soil, where generally the highest concentration of available carbon is present (Rolston, 1978). Weier *et al.* (1993b) reported that the N_2/N_2O ratio increases with increase in soil moisture and available carbon content. Addition of organic materials supplying available carbon to soil micro-organisms often results in enhanced denitrification (Feigin *et al.*, 1991).

Previous studies which examined the effect of wastewater irrigation on denitrification rates have produced several results and observations. Denitrification has been reported to be an important N removal process in land application of municipal wastewater (Ryden *et al.*, 1981; Monnett *et al.*, 1995; Sharpe and Harper, 1997). An increase in denitrification rate in a greenhouse experiment with a clay soil was detected under effluent irrigation (Feigin *et al.*, 1981). However, denitrification rates decreased within 1 to 2 days after irrigation with large amounts of effluent containing low levels of NO_3^- -N (Ryden *et al.*, 1981). Feigin and Kipnis (1980) observed that an increase in irrigation frequency in a field of Rhodes grass under effluent irrigation led to an increase in soil organic carbon content. As a result, N losses increased. The maximum denitrification rates from the upper soil surface layer have been observed in the region closest to the outflow of a constructed wetland (Fey *et al.*, 1999).

In this study, an experiment was conducted to evaluate the effect of a four-year irrigation period either with secondary wastewater or well water on total denitrification rates from the topsoil when nitrate and a source of carbon were applied to the soil samples.

5.1.1. Overall hypothesis

Null hypothesis - there is no difference in total denitrification between soil irrigated with wastewater and soil irrigated with well water.

$$H_0 \quad \mu_1 = \mu_2$$

Alternative hypothesis - there is a difference in total denitrification between these two irrigated soils.

$$H_1 \quad \mu_1 \neq \mu_2$$

This alternative hypothesis was that greater denitrification rates would be expected to occur in the soil irrigated with wastewater, due to the high BOD₅ of wastewater and high exchangeable sodium percentage (ESP) which results in soil structure breakdown.

5.2 Materials and methods

5.2.1 Design and treatments

A 2³ factorial experiment with 3 replications was employed. The experiment layout is shown in Table 5.1. The experimental factors were as follows:

Factor A: Irrigated soil quality (quality)

Q1= Soil irrigated with wastewater.

Q3= Soil irrigated with fresh water (well water).

Factor B: Irrigation water quality and method of irrigating repacked soil cores before incubation (crust formation)

Crusted: irrigated with distilled water using a rain simulator to maximize crust formation.

Uncrusted: irrigated manually with 0.1 M CaCl₂ solution to prevent clay dispersion and minimize crust formation

Factor C: Soil core moisture status before incubation (moisture)

Moist: drained for two days after irrigation

Wet: drained for two hours after irrigation

Table 5.1 Summary of treatments and experiment layout.

Soil quality	Crusted		Uncrusted	
	Irrigated by distilled water using rain simulator		Irrigated manually by 0.1 M CaCl ₂	
	Moist	Wet	Moist	Wet
Q1				
soil irrigated with wastewater	T1	T2	T3	T4
Q3				
soil irrigated with well water	T5	T6	T7	T8

where

T1 = Soil cores irrigated with wastewater in the field and with distilled water using a rain simulator (crusted) at moist soil moisture status before incubation.

T2 = Soil cores irrigated with wastewater in the field and with distilled water using a rain simulator (crusted) at wet soil moisture status before incubation.

T3 = Soil cores irrigated with wastewater in the field and with 0.1 M CaCl₂ solution manually (uncrusted) at moist soil moisture status before incubation.

T4 = Soil cores irrigated with wastewater in the field and with 0.1 M CaCl₂ solution manually (uncrusted) at wet soil moisture status before incubation.

T5 = Soil cores irrigated with well water in the field and with distilled water using a rain simulator (crusted) at moist soil moisture status before incubation.

T6 = Soil cores irrigated with well water in the field and with distilled water using a rain simulator (crusted) at wet soil moisture status before incubation.

T7 = Soil cores irrigated with well water in the field and with 0.1 M CaCl_2 solution manually (uncrusted) at moist soil moisture status before incubation.

T8 = Soil cores irrigated with well water in the field and with 0.1 M CaCl_2 solution manually (uncrusted) at wet soil moisture status before incubation.

The fresh loose topsoil samples (0-5 cm depth) were collected from six plots located in the Galicos River site in Greece at the end of the irrigation season (August 2000). Three plots had been irrigated with fresh (well) water and the other three plots with wastewater over four years, using a furrow irrigation system. The collected soil samples were repacked in metal rings (5 cm length, 7.3 cm diameter). Specifically, an amount of fresh soil equivalent to 244.8 g dry soil was repacked in each metal ring using an oedometer, and packed to a dry bulk density of 1.3 g cm^{-3} . A space of 0.5 cm was left above the surface of the soil cores in the metal rings, necessary for the irrigation water and nitrogen solution application, leaving cores of 4.5 cm in length.

A total of thirty repacked topsoil cores were prepared. Prior to core irrigation, each soil core was placed over a fine-gauge nylon mesh fabric and secured with super glue to prevent soil from falling out during irrigation and incubation. The properties of the soil samples used in these repacked cores are given in Table 5.2. These properties were measured before incubation.

The soil cores were then placed over a layer of coarse sand. Then using the volume of dry soil in the repacked cores and their field moisture content, the amount of irrigating water required to reach the air-filled porosity of $0.05 \text{ cm}^3 \text{ pores cm}^{-3}$ soil in each soil core was calculated. 10 ml was then subtracted from this volume, to be added in the form of a nitrogen fertilizer and glucose solution. The adjusted volume of irrigation water corresponded to 53 and 61 ml of either distilled water or CaCl_2 solution per core for the wastewater and well water treatments respectively.

A total of twenty four out of thirty soil cores (eight treatments \times three replicates) were used to test the effect of these treatments on total denitrification rates. The adjusted volume of distilled water was applied to six cores of the wastewater-treated

soil cores and to six of the well water soil cores. Distilled water was applied using a rainfall simulator maintained at a height of 60 cm above the soil core (“crusted” treatment). The other twelve soil cores (six from wastewater-irrigated soil and six from well water-irrigated soil) received 0.1 M CaCl₂ applied manually (“uncrusted” treatment). From each set of six cores that received the adjusted volume of distilled water or calcium chloride solution, three cores were watered two days prior to incubation (“moist treatment”), the other three cores two hours before incubation (“wet treatment”). After watering, these cores were allowed to drain over a thin layer of coarse sand at room temperature. Before incubation, irrigated soil cores were reweighed to calculate the amount of water remaining in each core, in order to derive the total head space volume of the incubation system. Then 10 ml of glucose and ¹⁵N-labelled nitrogen fertilizer solution (5 g l⁻¹ glucose and 150 mg N l⁻¹ prepared from 99.67 atoms percent calcium nitrate) was added to each core. These amendments were equivalent to 6 µg N g⁻¹ dry soil and 81.5 µg C g⁻¹ dry soil. The initial water-soluble organic carbon (WSOC) concentration in the topsoil irrigated either with wastewater or well water in the field and used in these repacked cores averaged 45 and 34 µg C g⁻¹ dry soil respectively (Table 5.2). Since concentrations of 40-80 µg C g⁻¹ dry soil have been reported to be necessary before denitrification is observed in soils (Beauchamp et al., 1980), it was decided to add carbon in the form of glucose (5 g l⁻¹). This was intended to provide sufficient soluble carbon to act as an energy source for denitrifiers.

The distilled water used for the preparation of the glucose and fertilizer solution was acetylated by passing acetylene gas through cold distilled water for 10 minutes. This was to carry acetylene more efficiently into the soil pores along with the nitrogen solution (Bragan *et al.*, 1997).

To the remaining six of the thirty repacked soil cores, three from wastewater and three from well water-irrigated soil at their field moisture contents of $\theta = 0.138$ and 0.079 respectively, 10 ml of labelled ¹⁵N nitrogen fertilizer solution without glucose (150 mg N l⁻¹ prepared from 99.67 atoms percent calcium nitrate) was added to each core. These cores represent the control treatments: C-S, control for wastewater

treatment and C-W, control for well water treatment and were employed to estimate the percentage of ^{15}N recovery assuming no denitrification losses occurred.

Table 5.2 Physical and some chemical properties of topsoils irrigated either with wastewater (Q1) or well water (Q3). Figures represent average \pm s.d (n=3).

Irrigated soil quality	Soil irrigated with wastewater			Soil irrigated with well water		
	Plot-1	Plot-2	Plot-3	Plot-4	Plot-5	Plot-6
pH	8.7 \pm 0.02	8.6 \pm 0.015	8.7 \pm 0.02	8.3 \pm 0.012	8.4 \pm 0.02	8.3 \pm 0.03
EC (dS m ⁻¹)	2.20 \pm 0.17	2.80 \pm 0.25	2.70 \pm 0.23	0.55 \pm 0.05	0.50 \pm 0.01	0.56 \pm 0.02
Soluble cations (mg l ⁻¹)						
Na ⁺	463 \pm 7.50	458 \pm 4.00	448 \pm 4.04	40 \pm 2.51	44 \pm 2.00	49 \pm 2.08
K ⁺	12.0 \pm 0.72	13.6 \pm 0.52	10.1 \pm 0.43	4.10 \pm 0.15	5.00 \pm 0.15	4.80 \pm 0.11
Ca ⁺⁺	43.0 \pm 1.73	39.0 \pm 4.58	45.0 \pm 1.50	62.0 \pm 2.08	65.3 \pm 1.52	60.7 \pm 1.69
Mg ⁺⁺	38.5 \pm 2.41	42.9 \pm 1.26	43.4 \pm 2.51	33.0 \pm 1.66	36.0 \pm 2.00	36.7 \pm 0.47
Soluble anions (mg l ⁻¹)						
Cl ⁻	60.4 \pm 2.01	64.1 \pm 1.15	67.0 \pm 1.10	2.10 \pm 0.05	2.95 \pm 0.150	3.04 \pm 0.26
HCO ₃ ⁻	12.3 \pm 0.35	12.0 \pm 0.19	12.6 \pm 0.21	8.00 \pm 0.15	7.50 \pm 0.25	8.10 \pm 0.30
Exchangeable cations (mmol _c 100 g ⁻¹ soil)						
Na ⁺	1.02 \pm 0.18	1.00 \pm 0.32	1.17 \pm 0.12	0.09 \pm 0.003	0.08 \pm 0.005	0.09 \pm 0.005
K ⁺	0.10 \pm 0.01	0.10 \pm 0.03	0.15 \pm 0.04	0.13 \pm 0.03	0.16 \pm 0.04	0.11 \pm 0.02
Ca ⁺⁺	2.90 \pm 0.20	3.00 \pm 0.20	2.97 \pm 0.15	4.80 \pm 0.20	4.40 \pm 0.17	4.00 \pm 0.30
Mg ⁺⁺	4.00 \pm 0.35	4.00 \pm 0.45	4.00 \pm 0.30	3.10 \pm 0.32	2.98 \pm 0.15	3.60 \pm 0.45
CEC (mmol _c 100 g ⁻¹ soil)	8.2 \pm 0.40	8.7 \pm 0.30	9.0 \pm 0.20	8.5 \pm 0.20	8.0 \pm 0.15	8.2 \pm 0.26
ESP (%)	12.4 \pm 0.64	11.5 \pm 1.13	13.0 \pm 1.09	1.05 \pm 0.13	1.00 \pm 0.12	1.09 \pm 0.16
SAR (mmol _c l ⁻¹)	12.3 \pm 0.36	11.9 \pm 0.13	12.1 \pm 0.37	1.01 \pm 0.10	1.08 \pm 0.07	1.20 \pm 0.02
Soil organic carbon (mg C g ⁻¹ dry soil)	9.90 \pm 0.36	10.0 \pm 0.27	10.2 \pm 0.20	10.0 \pm 0.50	9.00 \pm 0.26	9.80 \pm 0.20
Water-soluble organic carbon (µg C g ⁻¹ dry soil)	42.7 \pm 2.51	48.0 \pm 2.51	44.0 \pm 3.60	34 \pm 2.51	37.0 \pm 1.73	32.2 \pm 2.00
NH ₄ ⁺ -N (µg g ⁻¹ dry soil)	0.10 \pm 0.02	0.09 \pm 0.01	0.10 \pm 0.01	0.09 \pm 0.02	0.05 \pm 0.02	0.09 \pm 0.01
NO ₃ ⁻ -N (µg g ⁻¹ dry soil)	1.70 \pm 0.20	1.70 \pm 0.30	2.04 \pm 0.30	0.80 \pm 0.15	1.01 \pm 0.10	0.95 \pm 0.03
CaCO ₃ (%)	3.60 \pm 0.38	4.00 \pm 0.32	4.10 \pm 0.29	2.20 \pm 0.15	2.20 \pm 0.10	2.00 \pm 0.11
Sand (%)	25.0 \pm 3.21	25.9 \pm 3.51	27.0 \pm 2.02	38.0 \pm 3.60	40 \pm 2.00	41.0 \pm 0.76
Silt (%)	51.6 \pm 2.51	50.6 \pm 2.05	51.0 \pm 1.47	48.0 \pm 2.08	46.0 \pm 2.51	45.1 \pm 2.00
Clay (%)	23.0 \pm 2.02	22.6 \pm 2.07	22.0 \pm 1.47	13.5 \pm 0.89	13.9 \pm 0.87	13.0 \pm 0.90
Hydraulic conductivity (cm h ⁻¹)	0.93 \pm 0.05	0.86 \pm 0.04	1.05 \pm 0.09	1.00 \pm 0.03	1.12 \pm 0.02	1.10 \pm 0.04

5.2.2 Closed incubation system and incubation of the repacked soil cores

Tedlar bags were fixed to both ends of the incubation system (Figure 5.1). Using a syringe, the upper bag was inflated with a mixture of 90 ml of air and 10 ml of acetylene (10 % C_2H_2 by volume). The total pore volume in the repacked cores was 95 cm^3 , calculated using the total soil porosity and the volume of the dry soil in the repacked cores. The air-acetylene mixture in the upper filled bag was to replace the air displaced by the percolating fertilizer solution. The lower bag was left empty and open to equilibrate the air moving in and out during percolation of nitrogen fertilizer solution through the soil core. From the head space, 30 ml of air was withdrawn using a syringe and replaced by 30 ml of acetylene (approximately 10 % by volume), then the system was incubated at $15\text{ }^{\circ}\text{C}$ throughout the experimental period except for sampling times (a few minutes for each sampling).

5.2.3 N-gaseous emission measurement apparatus

N_2 emission during denitrification is difficult to measure. This is because its background level in the atmosphere is very high. Researchers commonly overcome this problem by inhibiting further reduction of N_2O to N_2 gas using acetylene (Yoshinari *et al.*, 1977) and then analyzing N_2O using a gas chromatograph (GC) such as the Hewlett Packard, Model 5890 Series II used in this project, equipped with an electron capture detector (ECD). However, the inhibition may not always be effective. N_2O has a tendency to undergo further reduction to N_2 if the acetylene inhibition of N_2O reductase under more anaerobic conditions is not complete. Moreover, there are problems because of the transient nature of acetylene in the system, and its adverse effects on soil microbial metabolism under long-term studies (Focht, 1982). An alternative is to use isotope methods, involving labelling of nitrogen additions with ^{15}N . A mass spectrometer, such as the single inlet VG Isogas MM622 triple detector mass spectrometer, or a stable isotope ratio mass spectrometer (IRMS) can be used to measure total ^{15}N -enriched denitrified gas as $N_2 + N_2O$. However, the continuous-flow isotope-ratio mass spectrometer (CF-IRMS)

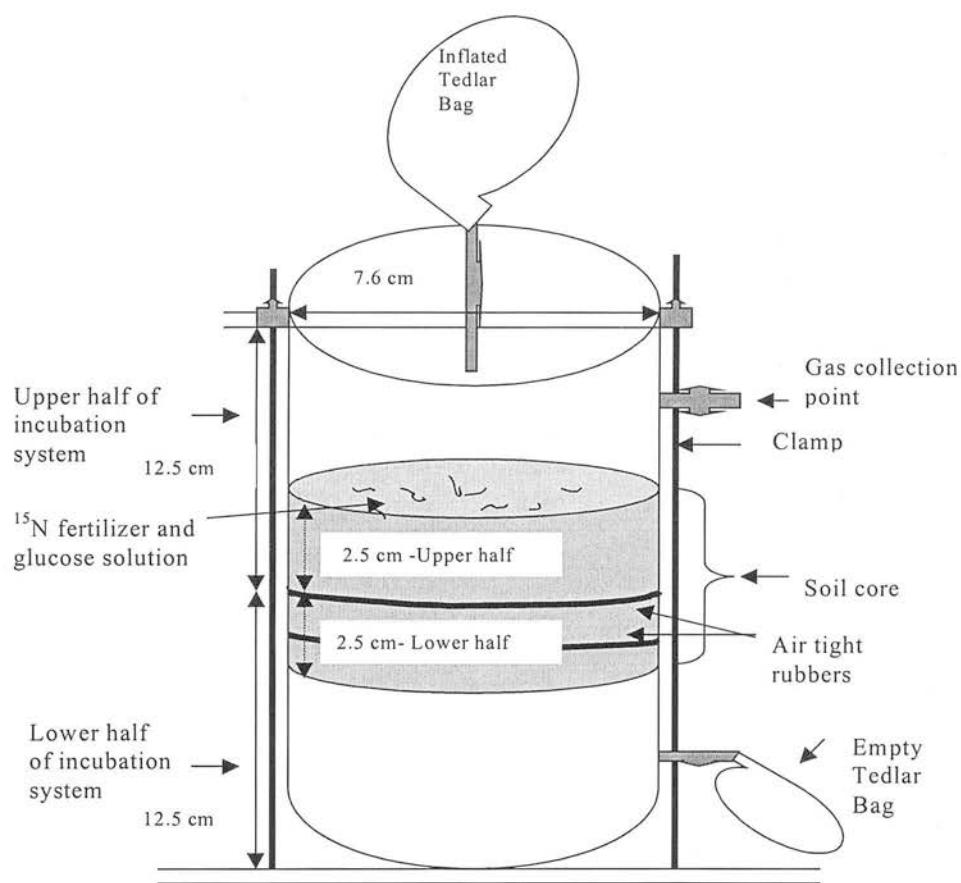


Figure 5.1 A representation of the closed incubation system used to investigate denitrification in irrigated topsoil cores. The two halves are isolated from each other by the air tight rubber.

is known to be accurate for measurements of the $^{15}\text{N}_2\text{O}$ fraction separately from the $^{15}\text{N}_2$ fraction (Stevens *et al.*, 1993).

At the Land Management Department at the Scottish Agricultural College (SAC) Edinburgh, a mass spectrometer that could be used for the direct measurement of ^{15}N -labelled gas emission was not available, and it would have been difficult to transport gas samples elsewhere for mass spectrometric analysis without contamination. Therefore, it was decided to study microbial reduction of nitrate in the top layer of irrigated soils using the acetylene inhibition method, estimating total denitrification by gas chromatographic analysis of nitrous oxide. ^{15}N -labelling was used to determine the recovery of the added nitrate.

5.2.4 Gas analysis

A gas chromatograph (GC) (Hewlett Packard, Model 5890 Series II) fitted with an electron capture detector (ECD) and thermal conductivity detector (TCD) was used to determine the concentrations of nitrous oxide (N_2O) and carbon dioxide (CO_2) respectively. The closed incubation system was connected to the GC by a metal tube at each sampling time (Plate 5.1). The results of experiments employing this analytical procedure already conducted on denitrification and respiration rates in soil aggregates can be seen in Appendices 2, and the results of those conducted on the effect of soil moisture status on denitrification and respiration rates in intact soil cores from the Galicos River site can be seen in Appendix 4. The first 10-ml gas samples were taken out automatically from the head space of the incubation systems after 24 hours of incubation and analyzed for CO_2 and N_2O . Then two more 10-ml gas samples were taken from the head space regularly every 24 hours and analyzed for N_2O and CO_2 . N_2O and CO_2 concentrations were determined by comparing the peak areas for samples and standards.

It was assumed that the diffusion into the lower and upper head spaces were the same. Therefore, it was decided to collect gas samples just from the upper head space. Using the acetylene inhibition method to estimate total denitrification in soil core incubations, there was a possibility of further reduction of nitrous oxide into

dinitrogen. To minimize this reduction, the water used for the preparation of the glucose and fertilizer solution was acetylated.

5.2.5 Infiltration rate and soil strength measurements

Following incubation, all soil cores were removed from the incubation systems. The depth at which the fertilizer solution penetrated the soil cores was not measured, but at the end of the incubation time, there was no fertilizer above the surface of the soil cores and none had drained from the soil cores. Because there was no solution drainage, all N_2O emitted was measured in the head space. The soil cores were placed in a refrigerator for two days over a thin layer of 70 grams of coarse sand, to remove any excess water. Then the surface layers were dried by circulating cold air in an oven for 24 hours. Soil strength was measured at several points using a drop cone penetrometer. However, an area of 11.34 cm^2 on each soil core surface was left intact to measure the surface infiltration rate.

Based on the principle of the single ring infiltrometer method (Bouwer, 1986), the surface infiltration rate in soil cores was measured using a small metal ring of 3.8 cm in diameter and 1 cm in height. The surface layer infiltration rate was estimated by measuring the time necessary for 11.3 cm^3 of water to percolate through the soil core surface.



Plate 5.1 The closed incubation system is shown together with the connected gas chromatograph equipment for measuring carbon dioxide (CO_2) and nitrous oxide (N_2O) concentrations in the head space.

5.2.6 Soil analysis

After measuring soil strength and infiltration rates, soils were removed from the metal ring, mixed with the 70 g of coarse sand which was used earlier to remove the excess of water, and left to air dry at room temperature for three days. Soils were then sieved (2 mm-mesh size), subsampled and analyzed for total nitrogen content according to the Kjeldahl method (Büchi Nitrogen-Information leaflet No.1) and also analyzed for ^{15}N contents. The ^{15}N contents were measured in the Land Management Department at the Scottish Agricultural College (SAC), Aberdeen using a mass spectrometer (MS) and the proportions of labelled and unlabelled nitrogen were calculated as follows:

$$\% \text{NDFF} = [(X - \text{N0} / \text{NF} - \text{N0}) \times (100/1)]$$

$$\% \text{NDFS} = [100 - \% \text{NDFF}]$$

where

$\% \text{NDFF}$ = % nitrogen derived from fertilizer

$\% \text{NDFS}$ = % nitrogen derived from soil

X = ^{15}N abundance of sample

N0 = Natural background ^{15}N abundance of 0.375 atom %

NF = ^{15}N abundance of fertilizer applied

5.2.7 Data analysis

The balanced factorial design was used to investigate the factors and their interaction effects on total denitrification rates, infiltration rate and soil strength of the repacked soil cores. Denitrification/respiration rates from the measured gas concentrations were calculated by subtracting the ambient N_2O and CO_2 concentrations from the concentration of gas in the closed system. N_2O and CO_2 fluxes were then calculated as follows:

$$\text{N}_2\text{O flux} = \left(\frac{(\text{ppm N}_2\text{O change} \times \text{Conversion factor} \times \text{Volume of head space})}{(\text{Soil sample dry weight} \times \text{time of incubation})} \right)$$

$$\text{CO}_2 \text{ flux} = \left(\frac{(\text{ppm CO}_2 \text{ change} \times \text{Conversion factor} \times \text{Volume of head space})}{(\text{Soil sample dry weight} \times \text{time of incubation})} \right)$$

Data from the experiment were prepared and tabulated in Excel and the means of replicates under each treatment with standard deviations were calculated. Denitrification rates were normalized by log-transformation (natural log) before statistical analysis (Parkin, 1993). In this balanced design, analysis of variance was carried out using the command for balanced ANOVA. The statistical analysis was conducted on mean denitrification/ respiration rates of days 1 and 2 of the experiment. Simple regression analysis was carried out to find out if there was any statistical relationship between air porosity, volumetric moisture content and volumetric moisture: air porosity ratio, and N₂O fluxes from the repacked soil core incubations. All statistical analyses were carried out using MiniTab 12.

5.3 Results

Total denitrification rates and CO₂ production during the incubation of irrigated soil cores, in the presence of 81.5 µg added C g⁻¹ dry soil are shown in Figures 5.2 and 5.3. The cumulative N₂O and CO₂ emissions are shown in Figures 5.4 and 5.5.

Under both irrigation water qualities, the highest N₂O fluxes under all treatments were observed after 2 days of incubation (Figure 5.2 a, b), after which they decreased on the final sampling.

Wastewater irrigation increased the total denitrification rates of the cores repacked with topsoil compared to those irrigated with well water, except from crusted cores at wet moisture status (T2) (Table 5.1, Figure 5.2 a, b).

The maximum total denitrification rates observed in moist crusted and uncrusted soil cores from wastewater-irrigated plots, amended with 81.5 µg added carbon g⁻¹ dry soil, were 2123 and 2603 µg N kg⁻¹ dry soil day⁻¹ respectively, whereas maximum rates of 1643 and 1119 µg N kg⁻¹ dry soil day⁻¹ were observed from the corresponding soil cores from well water irrigated plots (Table 5.3).

Similarly, the highest CO₂ evolution rates from the soil cores were detected after two days' incubation (Figure 5.3a, b). CO₂ rates observed from crusted soil cores from either wastewater or well water irrigated plots were higher than those observed from uncrusted cores (Table 5.3, Figure 5.3a, b). The highest CO₂ production rates were not associated with the highest denitrification rates.

The maximum total denitrification rates observed for control treatments (C-S, C-W) were 2.3 and 1.85 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹ in soil core incubations irrigated either with wastewater or well water and incubated at their field moisture contents without carbon addition respectively (Table 5.4). The mean rate of days 1 and 2 of the experiment for total denitrification in amended soil cores from either wastewater or well water irrigated plots (Table 5.3) at moist and wet moisture status were higher under T1 (1398 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹) followed by T3 (1391 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹), T4 (1113 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹), T5 (994 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹), T6 (943 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹), T2 (830 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹), T8 (735 $\mu\text{g N kg}^{-1}$ dry soil), and T7 had the lowest (660 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹) (Table 5.3).

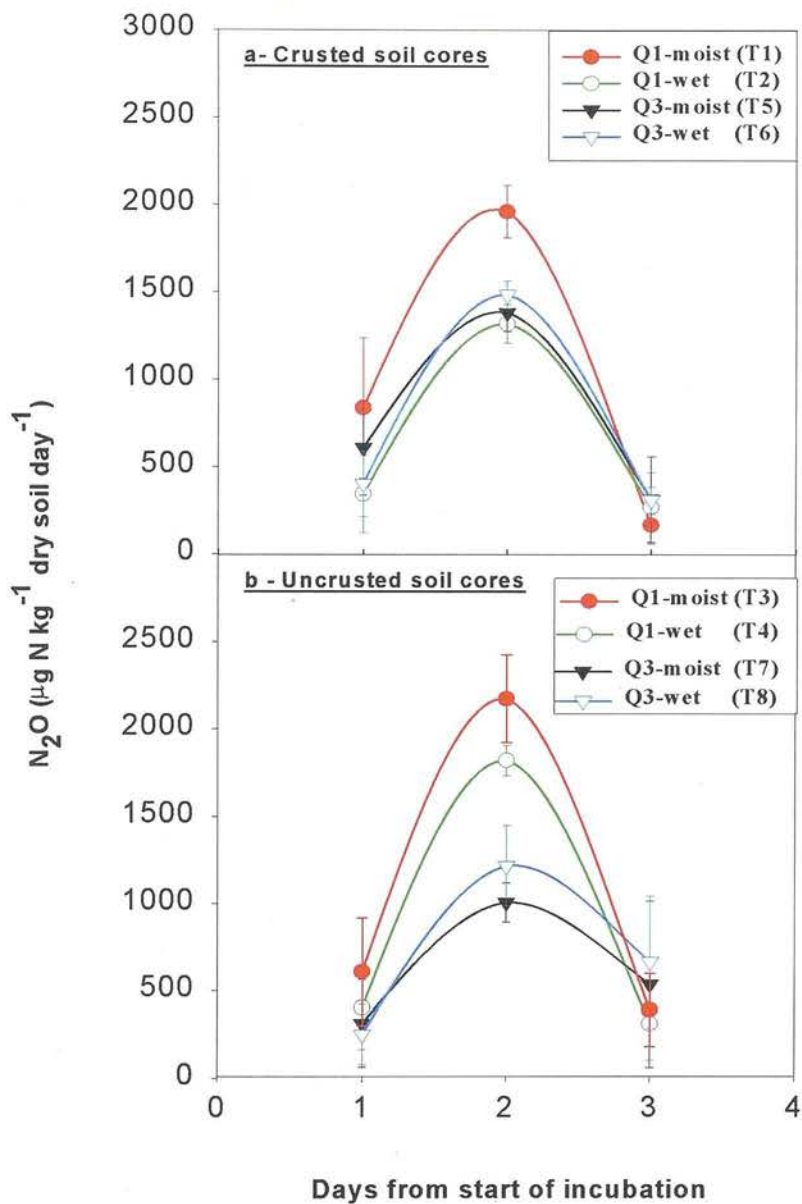


Figure 5.2 Denitrification rates of (a) crusted and (b) uncrusted repacked topsoil cores irrigated either with wastewater (Q1) or well water (Q3) at moist and wet soil moisture status before incubation, after addition of $81.5 \mu\text{g C g}^{-1}$ dry soil. Error bars represent standard deviation of the respective mean of three replicates.

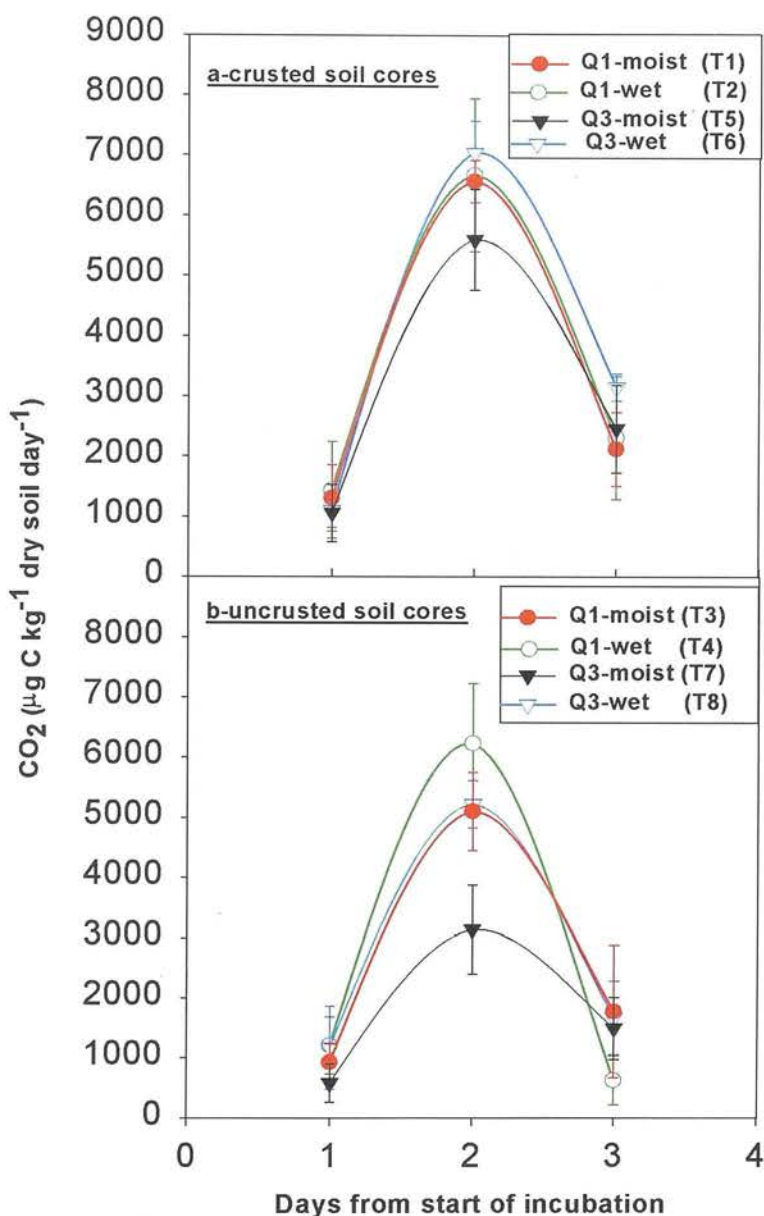


Figure 5.3 Carbon dioxide (CO_2) production as measured by thermal conductivity-gas chromatography in (a) crusted and (b) uncrusted soil cores of wastewater (Q1) and well water (Q3) irrigated soil at moist and wet soil moisture status before incubation, after addition of $81.5 \mu\text{g C g}^{-1}$ dry soil. Error bars represent standard deviation of the respective mean of three replicates.

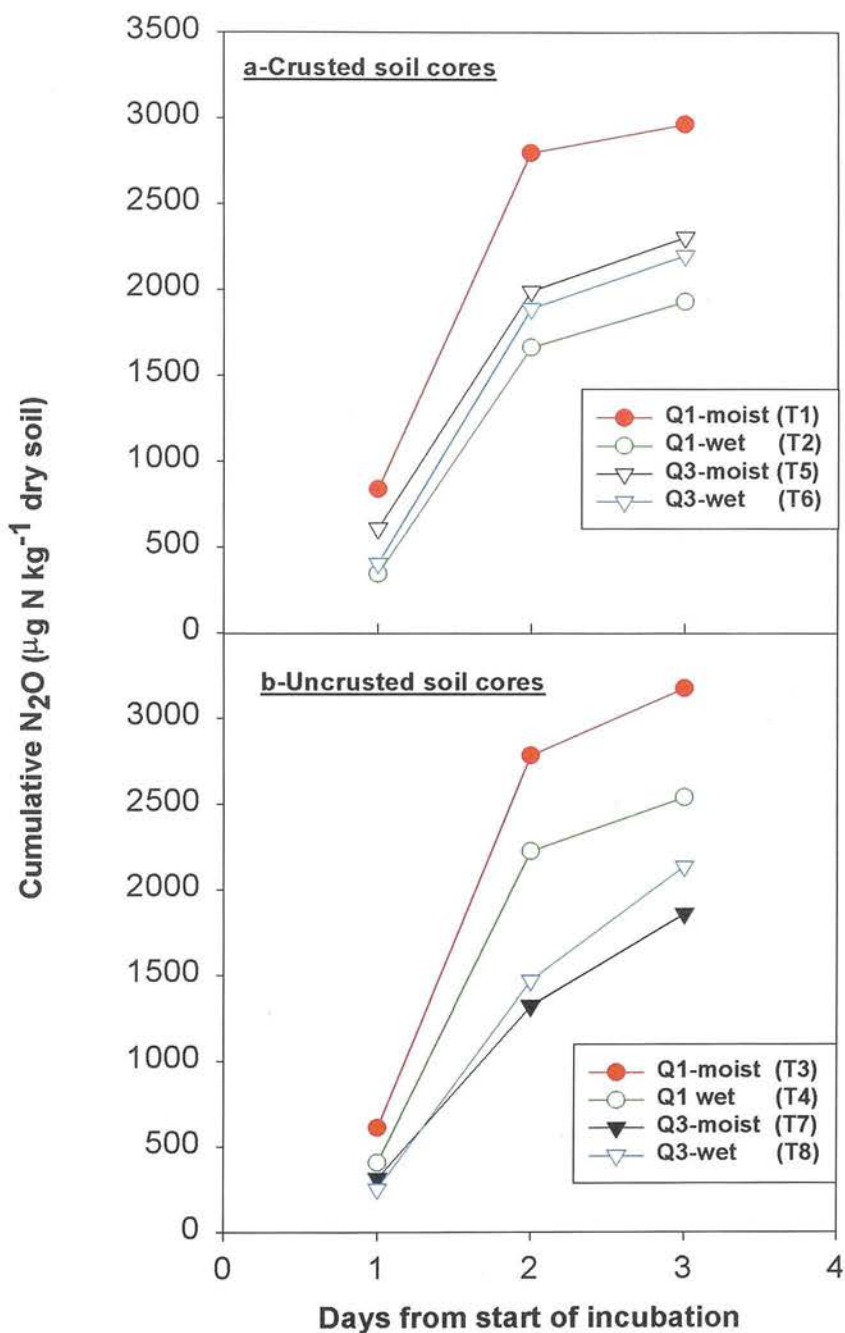


Figure 5.4 Cumulative N_2O emitted from incubations of (a) crusted and (b) uncrusted repacked topsoil cores irrigated either with wastewater (Q1) or well water (Q3) at moist and wet soil moisture status before incubation, after addition of $81.5 \mu\text{g C g}^{-1}$ dry soil.

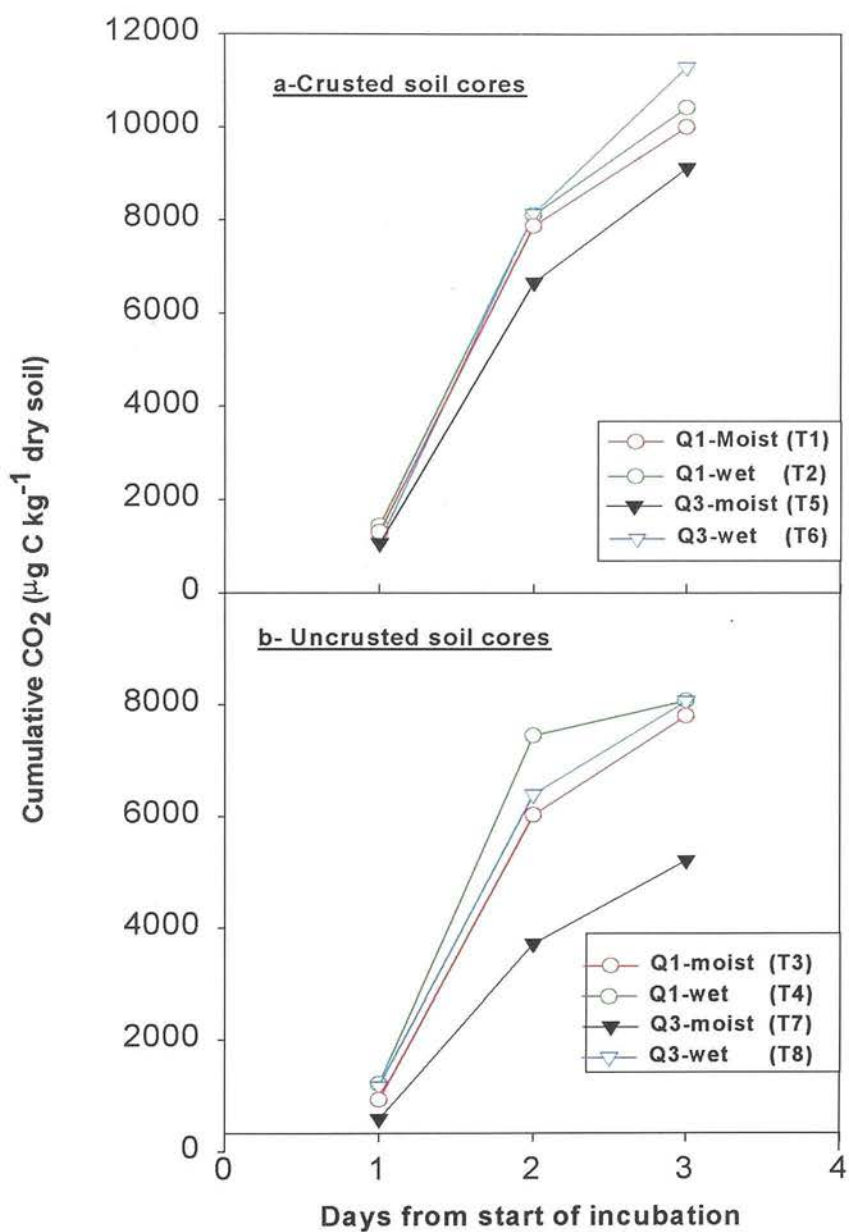


Figure 5.5 Cumulative CO₂ emitted from incubations of (a) crusted and (b) uncrusted repacked topsoil cores irrigated either with wastewater (Q1) or well water (Q3) at moist and wet soil moisture status before incubation, after addition of 81.5 $\mu\text{g C g}^{-1}$ dry soil.

Table 5.3 Maximum of the individual values and mean rates of days 1 and 2 of the experiment for total denitrification ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day $^{-1}$) and carbon dioxide production ($\mu\text{g CO}_2\text{-C kg}^{-1}$ dry soil day $^{-1}$) measured using the acetylene inhibition method and estimates of the total denitrification by gas chromatographic analysis of nitrous oxide in carbon amended soil cores.

Irrigated soil quality	Soil core moisture status before incubation	Denitrification ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day $^{-1}$)				CO ₂ production ($\mu\text{g CO}_2\text{-C kg}^{-1}$ dry soil day $^{-1}$)			
		Crusted		Uncrusted		<i>Crusted</i>		<i>Uncrusted</i>	
		Max	Mean	Max	Mean	<i>Max</i>	<i>Mean</i>	<i>Max</i>	<i>Mean</i>
(Q1) Wastewater irrigated soil	Moist	T1		T3		T1		T3	
		2123	1398	2603	1391	6948	3937	5750	3011
	Wet	T2		T4		T2		T4	
		1776	830	1882	1113	8222	4055	7748	3721
(Q3) well water irrigated soil	Moist	T5		T7		T5		T7	
		1643	994	1119	660	6655	3326	4499	1856
	Wet	T6		T8		T6		T8	
		1552	943	1376	735	7646	4068	5442	3194

where

T1-T8 as specified in materials and methods (section 5.2.1, Table 5.1)

Table 5.4 Maximum of the individual values and mean rates of days 1 and 2 of the experiment for total denitrification ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day^{-1}) and carbon dioxide production ($\mu\text{g CO}_2\text{-C kg}^{-1}$ dry soil day^{-1}) measured using the acetylene inhibition method and estimates of total denitrification by gas chromatographic analysis of nitrous oxide in soil cores used under control treatments.

Irrigated soil quality	Soil core moisture status before incubation	Denitrification ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day^{-1})		CO ₂ production ($\mu\text{g CO}_2\text{-C kg}^{-1}$ dry soil day^{-1})	
		Max	Mean	Max	Mean
Q1) Wastewater irrigated soil (C-S)	Field moisture	C-S	C-S	C-S	C-S
		2.3	1.63	3119	2304
(Q3) well water irrigated soil (C-W)	Field moisture	C-W	C-W	C-W	C-W
		1.85	1.26	2810	1565

The significance of differences between the total denitrification rates detected from core incubations from either wastewater or well water-irrigated soils without C amendment (control treatments), and those from C-amended cores was evaluated using one way analysis of variance. The total denitrification rates of soil cores from either wastewater or well water irrigated plots under control treatments were significantly lower than those under C-amendment treatments ($F_{9, 29} = 16.30$, $P < 0.001$). However, no significant difference between the total denitrification rates of the wastewater and the well water-irrigated soil core incubations under control treatments was detected.

Table 5.5 presents the results of analysis of variance for the factors (irrigated soil quality, crust formation and soil moisture status before incubation) and their interactions affecting mean denitrification rates ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day^{-1}) on days 1 and 2 of the experiment, for incubations of repacked soil cores amended with $81.5 \mu\text{g C g}^{-1}$ dry soil. The analysis indicated that the total denitrification rates were very significantly ($p < 0.05$) affected by the irrigated soil quality in the field (Q1, Q3). Also, the effects of crust formation and soil moisture status before incubation were significantly ($p < 0.05$) dependent on the quality of irrigation water in the field. However, the method of irrigating repacked soil cores before incubation (crusted, uncrusted) and the soil moisture status of cores before incubation (moist, wet) had no significant effect on total denitrification rates. Moreover, the carbon dioxide production rates from the same soil cores were significantly ($p < 0.05$) affected by the irrigation water quality in the field (Q1, Q3) and soil moisture status before incubation (moist, wet), and highly significantly ($p < 0.01$) affected by the crust formation (crusted, uncrusted) (Table 5.6).

The volumetric moisture: air-filled porosity ratio ($V_m:Ap$) of soils can be considered to be more important than just air porosity (Ap) in influencing denitrification in soil, as it gives an indication of the extent of anoxic conditions within the soil. As the $V_m:Ap$ ratio increases, more soil pores fill with water, therefore diffusion of O_2 into the soil decreases, resulting in an increase in anoxic microsites. The $V_m:Ap$ ratios were similar for both irrigated soil qualities.

The $V_m:Ap$ ratio method of considering gaseous diffusion in the soil was compared with N_2O emissions for the same incubations (Figure 5.6 a, b), and simple regression analysis was carried out to investigate relationships between air porosity, volumetric moisture content and the $V_m:Ap$ ratio, and total denitrification rates in the crusted and uncrusted soil cores (Table 5.7). No statistically significant relationship was found between denitrification rates and air porosity, volumetric moisture content and the volumetric moisture:air porosity ratio, since all R^2 values were $< 41\%$ for all experimental soil core incubations (Figure 5.6 a, b, Table 5.7).

Table 5.5 Analysis of variance for different sources of variation for mean denitrification rates ($\mu\text{g N}_2\text{O-N kg}^{-1} \text{ dry soil day}^{-1}$) of days 1 and 2 of the experiment at repacked soil core incubations after addition of $81.5 \mu\text{g C g}^{-1}$ dry soil. NS = not significant at $p>0.05$; * = significant at $p<0.05$; *** = significant at $p<0.001$.

Source of variation	DF	SS	MS	Significance
Irrigated soil quality (Q1, Q3)	1	0.134045	0.134045	***
Crust formation(crusted,uncrusted)	1	0.010248	0.010248	NS
Moisture (Moist, Wet)	1	0.030256	0.030256	NS
Soil quality*Crust formation	1	0.073965	0.073965	*
Soil quality*Moisture	1	0.040834	0.040834	*
Moisture*Crust formation	1	0.012396	0.012396	NS
Soil quality*Crustformation*Moisture	1	0.000859	0.000859	NS
Error	16	0.140416	0.008776	
Total	23	0.443018		

Table 5.6 Analysis of variance for different sources of variation for the mean carbon dioxide production ($\mu\text{g CO}_2\text{-C kg}^{-1} \text{ dry soil day}^{-1}$) for days 1 and 2 of the experiment at repacked soil core incubations after addition of $81.5 \mu\text{g C g}^{-1}$ dry soil. NS = not significant at $p>0.05$; * = significant at $p<0.05$; ** = significant at $p<0.01$.

Source of variation	DF	SS	MS	Significance
Irrigated soil quality (Q1, Q3)	1	1948563	1948563	*
Crust formation(crusted,uncrusted)	1	4868672	4868672	**
Moisture (Moist, Wet)	1	3170120	3170120	*
Soil quality*Crust formation	1	440671	440671	NS
Soil quality*Moisture	1	587425	587425	NS
Moisture*Crust formation	1	529182	529182	NS
Soil quality*Crustformation*Moisture	1	10	10	NS
Error	16	6958175	434886	
Total	23	18502819		

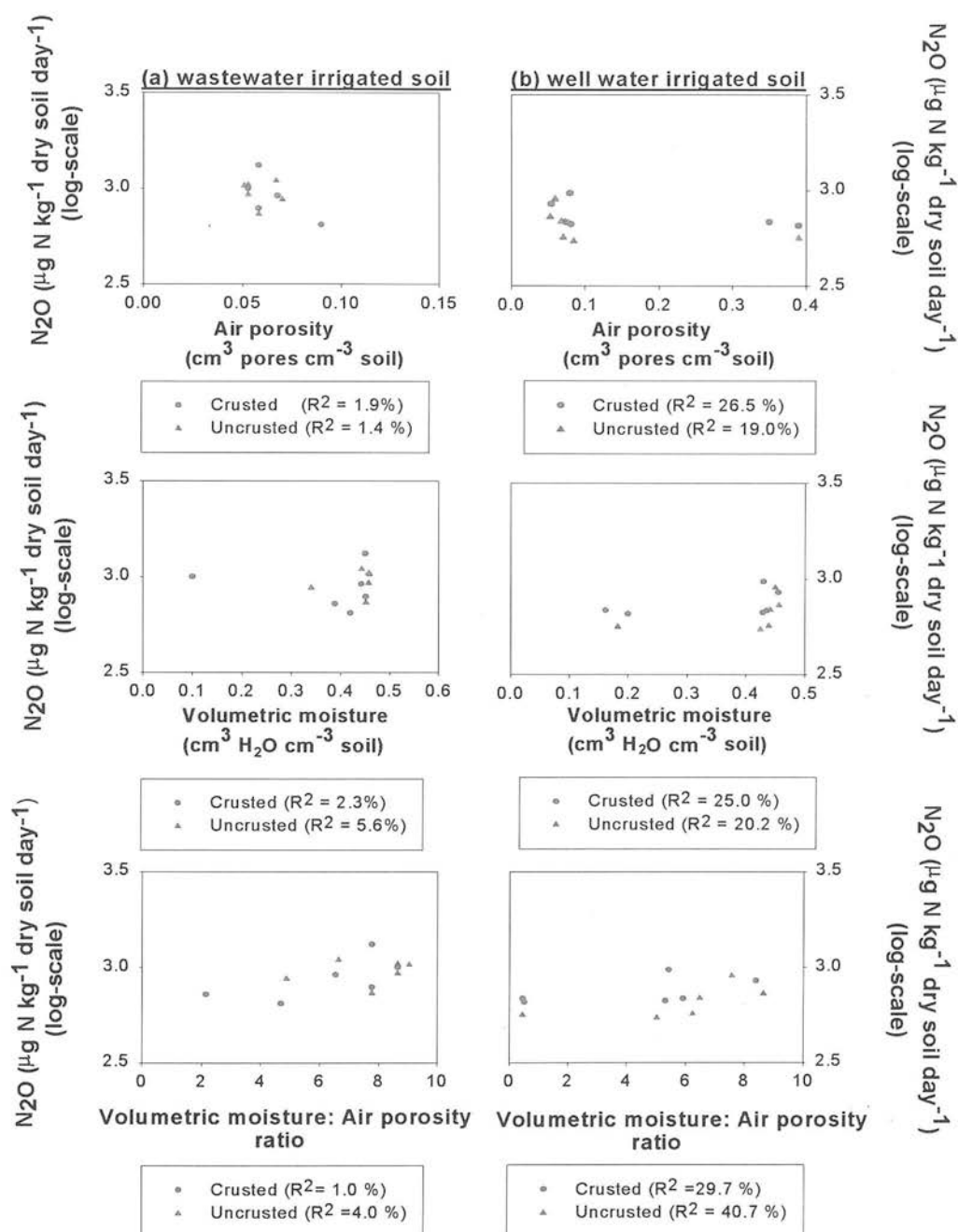


Figure 5.6 Observed effect of air-filled porosity, volumetric moisture and volumetric moisture:air porosity ratio on N₂O fluxes from soil cores irrigated with (a) wastewater and (b) well water after addition of 81.5 μg C g⁻¹ dry soil.

Table 5.7 Relationship between total denitrification rate (D , $\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day^{-1}) and (a) air porosity (A , cm^3 pores cm^{-3} soil), (b) volumetric moisture (V , $\text{cm}^3 \text{H}_2\text{O cm}^{-3}$ soil) and (c) Volumetric moisture: air porosity ratio (R) for soil core incubations from wastewater and well water-irrigated plots and either with distilled water applied using a rain simulator (crusted) or 0.1 M CaCl_2 solution applied manually (uncrusted) after addition of $81.5 \mu\text{g C g}^{-1}$ dry soil.

Soil quality	Regression equation	R^2 (%)
Wastewater-irrigated soil cores (Q1)		
- Crusted soil cores	(a) $D = 2.93 - 0.41A$	1.9
	(b) $D = 2.53 + 0.83V$	2.3
	(c) $D = 2.85 + 0.0058R$	1.0
- Uncrusted soil cores	(a) $D = 3.03 - 0.94 A$	1.4
	(b) $D = 2.83 + 0.327V$	5.6
	(c) $D = 2.91 + 0.0081R$	4.0
Well water-irrigated Soil cores (Q3)		
- Crusted soil cores	(a) $D = 2.91 - 0.230A$	26.5
	(b) $D = 2.78 + 0.258V$	25.0
	(c) $D = 2.82 + 0.0119R$	29.7
- Uncrusted soil cores	(a) $D = 2.85 - 0.282A$	19.0
	(b) $D = 2.67 + 0.361V$	20.2
	(c) $D = 2.71 + 0.0191R$	40.7

In order to obtain an indication of the accuracy of these measurements, a N balance for the soil core incubations was calculated (Table 5.8).

The highest nitrogen recovery was observed from soil cores under control treatments (C-S, C-W) at their field moisture content and without C amendment (Table 5.8). The recovery of the fertilizer N applied to the well water-irrigated cores and C amended (86.4 to 93.0 %) was higher than those from the corresponding wastewater-irrigated cores (55.3 to 86.9 %). Overall, 86.0 to 86.9 % (mean 86.4 %) of the fertilizer N applied to uncrusted repacked cores irrigated with wastewater (T3, T4) and 92.9 to 93.0 % (mean 92.9 %) applied to uncrusted soil cores irrigated with well water (T7, T8) was recovered at the end of the incubation period (Table 5.8). However, 55.3 to 73.7 % (mean 64.5 %) and 86.4 to 91.0 % (mean 88.7 %) of N applied to crusted soil cores irrigated either with wastewater (T1, T2) or well water (T5, T6) was recovered at the end of incubation, respectively (Table 5.8).

The percentage of nitrogen recovered from the crusted wastewater-irrigated soil cores at wet moisture status (T2) was lower than that estimated under moist conditions (T1) (Table 5.8).

The mean nitrogen recovery percentage (NR %) observed for soil cores irrigated with wastewater in the control treatment (C-S) was highest, followed by T4, T3, T1, with T2 the lowest. Moreover, the mean NR % for the soil cores irrigated with fresh water was highest in the control treatment (C-W), followed by treatments T8, T7, T6 and T5 which was the lowest (Table 5.8).

The mean $\text{N}_2\text{O-N}$ percentage lost from soil cores irrigated with wastewater was highest at T3, followed by T1, T4 and T2, with the control treatment (C-S) being the lowest. In addition, the mean $\text{N}_2\text{O-N}$ percentage lost from soil cores irrigated with well water was highest at T5, followed by T6, T8 and T7, with the control treatment (C-W) being the lowest (Table 5.8).

The mean ^{15}N % observed in soil cores irrigated with wastewater was highest in soil cores under the control treatment (C-S), followed by T4, T3 and T1 with T2 the lowest. ^{15}N % in soil was also highest in soil cores irrigated with well water under

the control treatment (C-W), followed by T7, T8 and T6, with T5 being the lowest (Table 5.8).

From the final nitrogen balance (Table 5.8), it was estimated that averages of 20 and 8 % of the nitrogen applied to wastewater and well water irrigated topsoil cores, respectively, under all treatments, in the presence and absence of added carbon, were unaccounted for at the end of incubation. Moreover, the average losses of nitrogen from the nitrogen fertilizer applied to these cores, and recovered as denitrified N_2O gas, were 35 and 28 %, respectively (Table 5.8).

Table 5.8 Nitrogen balance for core incubations of wastewater and well water-irrigated soils in the presence of 10 % (by volume) acetylene. Data shown are mean values for the three replicate cores.

Irrigated soil quality		Soil irrigated with wastewater					Soil irrigated with well water				
NO ₃ ⁻ added (μ g N g ⁻¹ dry soil)		6					6				
Treatments	Control (C-S)	T1	T2	T3	T4	Control (C-W)	T5	T6	T7	T8	
N balance (μg N)											
Fertilizer solution	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500
N ₂ O-N	1.057	725	472	777	621	0.86	564	538	455	523	
Final soil	1437	380	358	514	682	1475	732	828	939	872	
N unaccounted for	61.94	394	669	208	196	24.13	203	133	105	104	
N ₂ O-N %	0.07	48.39	31.49	51.82	41.45	0.05	37.60	35.87	30.34	34.87	
¹⁵ N % in final soil	95.80	25.33	23.86	34.26	45.46	98.33	48.80	55.20	62.60	58.13	
N- recovery %	95.87	73.72	55.35	86.08	86.91	98.39	86.40	91.07	92.94	93.00	
N % unaccounted for	4.12	26.27	44.64	13.91	13.08	1.60	13.59	8.92	7.05	6.99	

T1-T8 as specified in materials and methods (section 5.2.1, Table 5.1)

(C-S) = Soil cores irrigated with wastewater in the field and incubated at their field moisture content of $\theta = 0.138$ without carbon amendment and used as a control to estimate the percentage of ¹⁵N recovery in soil cores irrigated with wastewater assuming no denitrification losses.

(C-W) = Soil cores irrigated with well water in the field and incubated at their field moisture content of $\theta = 0.079$ without carbon amendment and used as a control to estimate the percentage of ¹⁵N recovery in soil cores irrigated with well water assuming no denitrification losses.

The soil infiltration rate (IR) for the crusted C-amended cores at the end of incubation was lower than the IR in the uncrusted C-amended cores (Figure 5.7a). However, the soil strength was higher in the crusted cores (Figure 5.7 b).

The order of the mean infiltration rates (cm h^{-1}) of irrigated soil cores after addition of $81.5 \mu\text{g C g}^{-1}$, from the lowest to the highest, were T2 (7.94) < T1 (10.23) < T6 (14.51) < T5 (15.14) < T3 (18.33) < T4 (18.65) < T8 (23.49) < T7 (25.67) (Table 5.9). However, the order of the means of soil strength (kg cm^{-2}) of irrigated soil cores from the lowest to the highest were T8 (9.53) < T7 (9.56) < T4 (10.03) < T6 (10.13) < T5 (10.30) < T3 (10.56) < T2 (11.03) < T1 (11.26) (Table 5.9).

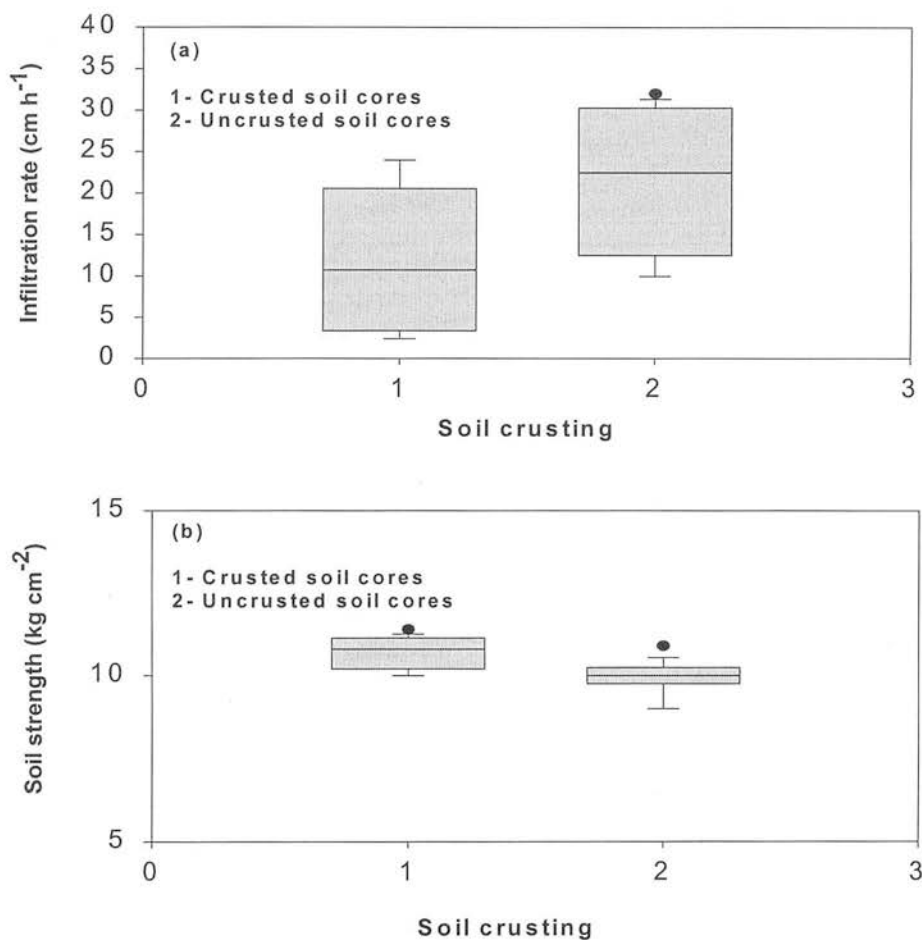


Figure 5.7 (a) surface layer infiltration rate and (b) soil strength at the end of incubation of (1) crusted and (2) uncrusted C-amended topsoil cores of soil irrigated either with wastewater or well water. T and ⊥ represent the positive and negative error bars of the respective means of twelve observations; • represent outliers and — (horizontal lines crossing the boxes) represent the median.

Table 5.9 Means \pm s.d of soil strength and infiltration rates of repacked C-amended soil cores from the Galicos River site irrigated either with wastewater or fresh water under different treatments (T1-T8) at the end of incubation.

Irrigated soil quality	Treatment Number	Soil strength (kg cm ⁻²)	Infiltration rate (cm h ⁻¹)
(Q1) soil irrigated with wastewater)	T1 (crusted)	11.26 \pm 2.11	10.23 \pm 3.06
	T2 (crusted)	11.03 \pm 1.05	7.94 \pm 2.10
	T3 (uncrusted)	10.56 \pm 3.28	18.33 \pm 3.80
	T4 (uncrusted)	10.03 \pm 2.05	18.65 \pm 4.10
(Q3) soil irrigated with well water	T5 (crusted)	10.30 \pm 2.30	15.14 \pm 3.0
	T6 (crusted)	10.13 \pm 2.11	14.51 \pm 2.60
	T7 (uncrusted)	9.56 \pm 2.51	25.67 \pm 4.04
	T8 (uncrusted)	9.53 \pm 1.46	23.49 \pm 5.20

Table 5.10 shows the statistical analysis of the effects of the main factors (irrigated soil quality, crust formation and soil moisture status before incubation) and their interactions in influencing IR of C-amended soil cores. This table shows that the infiltration rate in irrigated soil cores was significantly ($p<0.05$) affected by crust formation (the irrigation water quality and method of irrigating repacked soil cores before incubation). Also, the effect of crust formation on soil infiltration rate was not significantly dependent on the quality of irrigation water in the field and the soil moisture status before incubation (Table 5.10).

Table 5.10 Significant values of main factors and their interaction effect on infiltration rates (cm h^{-1}) of irrigated soil cores after addition of $81.5 \mu\text{g C g}^{-1}$ dry soil. NS = not significant at $p>0.05$; * = significant at $p<0.05$.

Source of variation	DF	SS	MS	Significance
Irrigated soil quality (Q1, Q3)	1	209.8	209.8	NS
Moisture (moist, wet)	1	8.5	8.5	NS
Crust formation (crusted, uncrusted)	1	550.3	550.3	*
Soil quality*Moisture	1	0.3	0.3	NS
Soil quality*Crust formation	1	0.2	0.2	NS
Moisture*Crust formation	1	0.4	0.4	NS
Soil quality*Moisture*Crust formation	1	6.5	6.5	NS
Error	16	1765.6	110.4	
Total	23	2541.7		

Table 5.11 shows results of the analysis of variance performed of the main factors and their interactions, in relation to soil strength (kg cm^{-2}) of the C-amended irrigated soil cores. The analysis indicates that the rates of soil strength in irrigated soil cores were very significantly ($p<0.001$) affected by irrigation water quality in the field (Q1, Q3) and crust formation (Table 5.11). However, there was no significant interaction effect among irrigation water qualities in the field (Q1, Q3), soil moisture status before incubation (moist, wet) and the crust formation treatment (crusted, uncrusted).

Table 5.11 Significance values of main factors and their interactions, in relation to soil strength (kg cm^{-2}) of the irrigated cores, after addition of $81.5 \mu\text{g C g}^{-1}$ dry soil. NS = not significant at $p>0.05$; *** = significant at $p<0.001$.

Source of Variance	DF	SS	MS	Significance
Irrigated soil quality(Q1, Q3)	1	4.2504	4.2504	***
Moisture (moist, wet)	1	0.3504	0.3504	NS
Crust formation(crusted, uncrusted)	1	3.4504	3.4504	***
Soil quality*moisture	1	0.1204	0.1204	NS
Soil quality*crust formation	1	0.0504	0.0504	NS
moistture*crust formation	1	0.0104	0.0104	NS
Soil quality*moisture*crust formation	1	0.0704	0.0704	NS
Error	16	1.3667	0.0854	
Total	23	9.6696		

5.4 Discussion

Soil irrigated with wastewater would be expected to have the highest denitrification levels both in the laboratory and in the field, due to the high biological oxygen demand (BOD_5) and nitrogen content of sewage water used for irrigation. The high exchangeable sodium percent (ESP) also has an impact on deterioration of soil structure through dispersion of clay particles, and consequently soil aeration and enhancing denitrification rates, compared to soil irrigated with well water.

Total denitrification rates observed in the finer textured wastewater-irrigated soil cores that had high SAR and ESP (Table 5.2) were significantly higher than those in coarser textured well water-irrigated soil cores. This might be attributed to the clay dispersion due to the high Na wastewater. Clay dispersion results in reduced hydraulic conductivity of the soil profile and infiltration rate and also leads to poor aeration and increased denitrification. Richards (1954) suggested that a SAR value above 10 and an ESP value of 15% are the critical levels above which soil structure is destroyed. However, Crescimanno *et al.* (1995) reported that significant soil degradation could occur even in a 2-5 ESP range at a low cationic concentration. As a result of the loss of structure, soil permeability problems occur, leading to poor aeration and increased nitrogen losses through denitrification. Another possible

explanation for the higher total denitrification rates observed in wastewater-irrigated soil cores is the natural difference in the clay content of the different parts of the site. The promotion of more anoxic zones in the smaller pores of finer textured wastewater-irrigated soil with increasing moisture and/or microbial CO_2 respiration. Assuming that this was occurring, this suggests that soil texture may have had an effect on topsoil denitrification during the period in which N_2O fluxes were measured. Results from work conducted by Lund *et al.* (1974) support the proposed explanation. However, Arah *et al.* (1991) reported that the N_2O fluxes measured from loam soil plots were higher than those from clay loam soil plots. This was attributed to further reduction of N_2O to N_2 in clay loam soil. Another possibility for the higher total denitrification rates under wastewater irrigation might be due to the high BOD_5 of wastewater. High BOD_5 reduces the oxygen level in the soil air, and consequently is likely to enhance denitrification.

The decrease in total denitrification rate observed in the final sampling data may have been due to the following reasons; (a) the alleviation of C_2H_2 inhibition of N_2O reductase and consequently the further reduction of N_2O to N_2 ; (b) reduction in microbial activity which could be due to the lack of carbon substrate that was added to the soil cores ($81.5 \mu\text{g C g}^{-1}$ dry soil) or due to the low levels of added $\text{NO}_3\text{-N}$ ($6 \mu\text{g N g}^{-1}$ dry soil); or (c) the leaking of the seals on the incubation systems.

The lowering in total denitrification rates observed in crusted cores from soil irrigated with wastewater under wet conditions (T_2) might be due to increased anoxic conditions which could cause a failure of the C_2H_2 inhibition of N_2O reductase in these cores and consequently, promoting further reduction of N_2O to N_2 . Moreover, a smaller particle size of wastewater-irrigated soil resulted in smaller pores which were more likely to become anoxic with increasing moisture and promote more anaerobic conditions. However, the differences in total denitrification rates between crusted and uncrusted soil cores were not statistically significant.

Non-statistical, curvilinear relationships have been observed between denitrification rates and air-filled porosity (A_p) (Burton and Beauchamp, 1985; Prade and Trolldenier, 1988). Similarly, in our study, no statistical relationship was detected

between A_p and total denitrification rates, thus indicating that A_p had no effect on denitrification in the repacked topsoil cores.

No statistical relationship was found between air-filled porosity, volumetric moisture content or the volumetric moisture: air porosity ratio and denitrification rates in the soil cores. This indicates that other factors rather than soil texture and gaseous diffusion were more important in limiting denitrification rates in these irrigated soils.

A highly significant increase in total denitrification rates registered for C-amended soil cores when compared with those of control treatments (C-unamended soil cores) indicates that available organic carbon was the main factor limiting denitrification in the repacked soil core incubations.

The percentage of nitrogen recovered from the crusted wastewater-irrigated soil cores at wet moisture status (T2) was lower than that estimated under moist conditions (T1). This was possibly due to the wet conditions causing less effective C_2H_2 inhibition of N_2O reductase. Therefore, in the wet treatment, the amount of N_2O reduced to N_2 would be higher than in the moist treatment. This confirms the work of Yoshinari *et al.* (1977) who reported a less effective inhibition of N_2O reductase in the wet state. Similarly, Focht (1974) reported that the more aerated the pore space in soil, the higher the percentage of N_2O in the denitrification gases was observed.

An average of 20 % of the nitrogen fertilizer applied to wastewater irrigated topsoil cores, and 8 % of the nitrogen fertilizer applied to well water irrigated topsoil cores were unaccounted for at the end of incubation. This suggests that there are other sinks of nitrogen in addition to those measured in incubations, such as further reduction of N_2O to N_2 .

Crust formation induced on the surface layer of repacked soil cores using a rain simulator increased soil strength, and subsequently significantly decreased the soil infiltration rate. However, its effect on total denitrification rates at soil cores was significantly dependent on the irrigated soil quality. Under crust formation

conditions, finer pore sizes in the surface layer resulted in higher water holding capacity (WHC) and promoted anaerobic conditions suitable for denitrification, but this was not occurring in the crusted soil cores under study. This was possibly because the crust layer induced on the surfaces of soil cores was not sufficient to inhibit aeration.

Potential denitrification rates of 2123 to 2603 and of 1776 to 1882 $\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day⁻¹ were observed in moist and wet crusted and uncrusted soil cores from plots under wastewater irrigation respectively, whereas the potential denitrification rates of 1119 to 1643 and of 1376 to 1552 $\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day⁻¹ were observed in moist and wet uncrusted and crusted cores from plots under well water irrigation respectively after adding 81.5 $\mu\text{g C g}^{-1}$ dry soil. Overall, these rates are lower than those reported in other studies looking at potential denitrification in C-amended topsoil using wastewater irrigation *versus* fresh water irrigation. Monnett *et al.* (1995) reported potential denitrification rates of 63000 to 66000 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹ in incubations of intact topsoil (0-7 cm) cores of silty clay texture irrigated with wastewater, compared with rates of 19200 $\mu\text{g N kg}^{-1}$ dry soil day⁻¹ observed from the same soil core incubations irrigated with potable water. The higher potential denitrification rates observed in Monnett's study might be due to the high carbon amendment (1800 $\mu\text{g C g}^{-1}$ dry soil) in combination with N amendment (84 $\mu\text{g NO}_3\text{-N g}^{-1}$ dry soil as KNO_3).

The observed reduction in the surface layer infiltration rate (IR) of crusted soil cores and the increase in soil strength compared with uncrusted cores is supported by the work of Tanaka *et al.* (1999). In their study they found that a large decrease in soil IR was caused by the action of rainwater, where crusting was evaluated in terms of the water permeability and morphological observation following the rainfall treatment. The comparative results on crusting strengths also agreed with those of Agassi *et al.* (1981), who worked with sodic soils in the laboratory. They found that at high soil ESP and low irrigation water salinity (rainwater), chemical dispersion processes have an increasing role in determining the infiltration rate of the soils. However, Wild (1988) reported that crusting strengths are related to the differences

in soil composition, structure and moisture content. Rainwater was found to have more deleterious effects than irrigation waters containing some salts (Oster and Schroer, 1979; Ayers and Westcot, 1985). Raindrop impact was found to be the main factor affecting aggregate disintegration through slaking (Tanaka *et al.*, 1999).

5.5 Overview

Irrigation treatments and available organic carbon were found to be the main factors limiting topsoil denitrification in incubated repacked soil cores from the study area. The higher total denitrification rates were observed in wastewater-irrigated soil cores, probably due to the natural differences in the clay content of the different parts of the site and the effect of higher sodium levels. The results presented here suggest that denitrifier populations in topsoils irrigated either with wastewater or well water in the presence of added carbon can be active, leading to significant levels of topsoil denitrification. This is likely to increase concerns over total atmospheric N₂O concentrations. Crust layers that had been induced on the surface layer of repacked soil cores had no significant effect on total denitrification rates. However, they decreased infiltration rate and increased soil strength significantly. The calculated average percentages of the nitrogen fertilizer applied to topsoil cores irrigated either with wastewater or well water that were unaccounted for at the end of incubation were 20 and 8 %, respectively.

6 Denitrification and available organic carbon in cores of intact topsoil

6.1 Introduction

Denitrification of nitrate by heterotrophic organisms cannot occur unless the substrate contains soil organic compounds which can support growth of the organisms and act as a hydrogen donor for the process of denitrification (Bremner and Shaw, 1958b). Denitrifying organisms use organic compounds as electron donors for energy and for synthesis of cellular constituents. Therefore, denitrification rates are strongly dependent on the amount of available organic carbon, its position in the profile and its position in relation to the NO_3^- in the soil (Rolston, 1981). Organic matter concentration tends to be greatest at the soil surface, therefore resulting in a decrease in denitrification potential down the soil profile (Rolston, 1981). Previous studies that looked at the effect of organic carbon on denitrification rates have reported that the soil content of available organic carbon is an important determinant for denitrification rate (Rolston, 1981; Weier *et al.*, 1993a).

Bremner and Shaw (1958b) showed that although denitrification was not detectable by total-N analysis when soils of low organic matter content were incubated with nitrate under waterlogged conditions, it was readily detectable when glucose was added or when soils of higher organic matter content were used.

Supply of organic matter can influence denitrification either directly as a growth substrate, or through increased microbial activity and O_2 consumption, resulting in the formation of anoxic microsites (Rolston, 1981). Subsequent rates of denitrification may also be influenced, as metabolisable C sources are required for the formation of adenosine triphosphate (ATP) from oxidative phosphorylation (Beauchamp *et al.*, 1989).

Incorporation of organic matter such as residues of straw or stubble into soil has been observed to increase denitrification rates, compared to rates in plots without

residue additions (Rolston *et al.*, 1982). The incorporation of crop residues results in the formation of “hotspots” (Parkin, 1987); within these “hotspots” high denitrifier activity can occur either within the organic substrate itself, or in the soil surrounding the particulate organic material. Burford and Bremner (1975) found that the denitrification capacities of 17 surface soils from Iowa were very highly correlated with water-soluble organic C or mineralizable C.

Sewage effluent resulting from domestic or industrial water use or produced by biological activity during secondary treatment contains an appreciable amount of organic matter (Hunter and Kotalik, 1974). The typical concentration of organic compounds in secondary wastewater ranges from 10 to 160 mg l⁻¹ (Thomas and Law, 1977; Idelovitch, 1978; Asano *et al.*, 1985) (see Table 2.1). Friedel *et al.* (2000) observed that long-term wastewater irrigation (up to 80 years) using water containing 99.3-161.8 mg l⁻¹ total organic matter increased the total organic carbon content of the surface soil (0-15 cm depth) 2.5-fold. As a result, the denitrification rate increased by 7 times compared with rates observed from the same soil not irrigated with wastewater.

The aim of the present study was to investigate the influence of available organic carbon added in the form of glucose on total denitrification rates when nitrate was applied to the soil samples. The soils studied were intact topsoil cores from either wastewater or well water irrigated plots over four years at the Galicos River Wastewater Treatment Project in Greece.

6.1.1 Overall hypothesis

The null hypothesis was that available organic carbon has no effect on denitrification, so that the denitrification rate from carbon-amended soil cores equals the denitrification rates from unamended soil cores.

The alternative hypothesis was that higher denitrification rates would be observed from carbon-amended soil cores. These would be attributable to the role of available-organic carbon in promoting denitrification, as it acts as an electron donor

(Tiedje *et al.*, 1982), or through increased heterotrophic respiration and O₂ consumption, resulting in the formation of anoxic microsites (Rolston, 1981).

6.2 Materials and methods

6.2.1 Design and treatments

A 2³ factorial experiment with two replications for carbon amendment treatments was designed. The layout of the experiment is shown in Table 6.1. The experimental factors were as in Chapter 5 (section 5.2.1). The exception was factor C as follows:

Factor C: Carbon amendments

- With carbon (+ carbon), (13.2 µg C g⁻¹ dry soil)
- Without carbon (-carbon)

Table 6.1 Summary of treatments and experiment layout

Irrigated soil quality	Crusted Irrigated by distilled water using rain simulator		Uncrusted Irrigated by 0.1M CaCl ₂ manually	
	(+ carbon)	(- carbon)	(+ carbon)	(- carbon)
Q1 (soil irrigated with wastewater)	^a T1+C ^b R=2	T1-C	T2+C R=2	T2-C
Q3 (soil irrigated with well water)	T3+C R=2	T3-C	T4+C R=2	T4-C

^aT= treatment

^bR= replicate

where

T1+C = Soil cores irrigated with wastewater in the field and with distilled water using a rain simulator (crusted) before incubation with carbon amendment addition (+ carbon).

T1-C = Soil cores irrigated with wastewater in the field and with distilled water using a rain simulator (crusted) before incubation without carbon amendment addition (- carbon) and used as a **control** for the T1+C treatment.

T2+C = Soil cores irrigated with wastewater in the field and with 0.1M CaCl₂ solution manually (uncrusted) before incubation with carbon amendment addition (+ carbon).

T2-C = Soil cores irrigated with wastewater in the field and with 0.1M CaCl₂ solution manually (uncrusted) before incubation without carbon amendment addition (- carbon) and used as a **control** for the T2+C treatment.

T3+C = Soil cores irrigated with well water in the field and with distilled water using a rain simulator (crusted) before incubation with carbon amendment addition (+ carbon).

T3-C = Soil cores irrigated with well water in the field and with distilled water using a rain simulator (crusted) before incubation without carbon amendment addition (- carbon) and used as a **control** for the T3+C treatment.

T4+C = Soil cores irrigated with well water in the field and with 0.1M CaCl₂ solution manually (uncrusted) before incubation with carbon amendment addition (+ carbon).

T4-C = Soil cores irrigated with well water in the field and with 0.1M CaCl₂ solution manually (uncrusted) before incubation without carbon amendment addition (- carbon) and used as a **control** for the T4+C treatment.

A number of intact soil cores (0-7.5 cm depth) were collected from different plots irrigated either with wastewater or well water over four years by a furrow at the Galicos River site in Greece at the beginning of the irrigation season (April 2000), and analysed for selected chemical and physical soil properties (results not shown). Based on these chemical and physical soil properties, four irrigated plots were selected, representative of the four treatments (T1, T2, T3 and T4) (Table 6.1). A

group of three intact soil cores (7.5 cm depth, 7.3 cm in diameter), two for C-amendment treatment, and one for non-C amendment treatment (control) were collected from each plot. Metal rings were hammered into the soil to a depth of 7.5 cm from the soil surface. Excess soil was removed from the bottom end of the ring. The bottom and the top of each core were sealed by a plastic lid to protect the soil core during transport, and to prevent evaporation. Then each core was sealed in polythene bag and stored at 4 °C prior to analysis and incubation. Additional intact soil cores were collected at the same depth from each plot and analyzed before incubation for soil electrical conductivity (EC), pH, exchangeable cations, cation exchange capacity, soil organic carbon content, mineral nitrogen and water-soluble organic carbon (WSOC). The topsoil properties are presented in Table 6.2.

Prior to irrigation, intact soil cores used in the incubations under study were allowed to slip down 1 cm. The 1 cm sections of soil that had slipped out of the bottom ends of the rings were cut off and measured for gravimetric moisture content. The 1 cm space that was left in the top of the metal rings was used to apply irrigation water and N fertilizer solution. The cores that were left were 6.5 cm in height and contained 353.7 g dry soil. To increase the air filled pore space (AFPS) in soil cores, the cores were partially dried by circulating cold air in an oven for 24 hours. Afterwards, the soil cores were reweighed, and the amount of water lost from each core was calculated. Soil cores were secured from the bottom ends using a piece of fine-gauge nylon mesh fabric. The adjusted volume of irrigating water or CaCl_2 solution required to reach the air filled porosity of $0.05 \text{ cm}^3 \text{ pores cm}^{-3} \text{ soil}$ in each core was calculated as described in Chapter 5 (section 5.2.1) and applied in the following ways:

1- Crusted: Three intact soil cores from wastewater, and three from well water-irrigated plots received 69 and 72 ml distilled water respectively. Distilled water was applied using rainfall simulator maintained at a height of 60 cm above the soil core ("crusted treatment"). In two of the cores from each group of three, the water was amended with glucose (equivalent to 59 mg C l^{-1}) in order to provide easily oxidizable carbon. This C-amendment resulted in an addition of carbon to soil cores

equivalent to an average of $11.5 \mu\text{g C g}^{-1}$ dry soil. The third core received distilled water only.

2- Uncrusted: The other six soil cores, three from wastewater and three from well water irrigated-plots were given 69 and 72 ml 0.1 M calcium chloride respectively. This amount was applied manually (“uncrusted treatment”), and similarly to the previous treatment, carbon (59 mg C l^{-1}) was added to two cores while the third core received CaCl_2 only.

The cores were left over a thin layer of coarse sand to allow the excess water to drain off for two days at room temperature. The cores were reweighed and the amount of water remaining in each core was calculated to derive the total head space volume of the incubation system. Then, 10 ml of glucose and labelled ^{15}N nitrogen fertilizer solution (59 mg C l^{-1} and 150 mg N l^{-1}) prepared from 99.67 atoms percent $\text{Ca}^{15}\text{NO}_3$ was added to each C-amended core, i.e. those which had received glucose (see above). The fertilizer solution, applied to the cores, gave a concentration of $4 \mu\text{g N g}^{-1}$ dry soil and $1.7 \mu\text{g C g}^{-1}$ dry soil.

The intact soil cores without C-amendment, i.e. those which did not receive any carbon before (see above), were treated with a fertilizer solution containing only 150 mg N l^{-1} and were used as controls. The distilled water, which was used for preparation of fertilizer solution, was acetylated as described in Chapter 5 (section 5.2.1).

Overall, each C-amended core received $11.5 \mu\text{g C g}^{-1}$ dry soil two days before incubation with nitrate through irrigation solution and $1.7 \mu\text{g C g}^{-1}$ dry soil provided during incubation with nitrate. The total amount of $13.2 \mu\text{g C g}^{-1}$ dry soil was used to investigate the effect of available organic carbon on total denitrification rates.

Table 6.2 Soil characteristics of the topsoils irrigated either with wastewater (Q1) or well water (Q3) at the Galicos River Wastewater Treatment Project before incubation. Figures represent the average \pm s.d (n=3).

Parameter	Soil irrigated with wastewater		Soil irrigated with well water	
	Plot-1	Plot-2	Plot-3	Plot-4
pH	8.6 \pm 0.02	8.7 \pm 0.03	8.4 \pm 0.01	8.4 \pm 0.02
EC (dS m ⁻¹)	1.07 \pm 0.09	0.91 \pm 0.07	0.50 \pm 0.03	0.48 \pm 0.04
Exchangeable cations (mmol _c 100g ⁻¹ soil)				
Na ⁺	0.83 \pm 0.10	0.70 \pm 0.12	0.09 \pm 0.003	0.08 \pm 0.002
K ⁺	0.10 \pm 0.01	0.15 \pm 0.02	0.13 \pm 0.04	0.16 \pm 0.03
Ca ⁺⁺	2.94 \pm 0.22	3.12 \pm 0.20	4.8.0 \pm 0.12	4.40 \pm 0.14
Mg ⁺⁺	4.34 \pm 0.28	4.00 \pm 0.30	3.10 \pm 0.10	2.98 \pm 0.15
CEC (mmol _c 100 g ⁻¹ soil)	8.60 \pm 0.38	8.81 \pm 0.41	8.50 \pm 0.15	8.00 \pm 0.20
ESP (%)	9.65 \pm 0.53	8.96 \pm 0.41	1.05 \pm 0.15	1.00 \pm 0.13
Soil organic carbon (mg C g ⁻¹ dry soil)	10.0 \pm 0.24	9.92 \pm 0.31	9.52 \pm 0.20	10.01 \pm 0.26
Water-soluble organic carbon (μ g C g ⁻¹ dry soil)	42.18 \pm 1.60	45.60 \pm 2.04	35.20 \pm 1.12	31.66 \pm 1.20
NH ₄ ⁺ -N (μ g g ⁻¹ dry soil)	0.11 \pm 0.02	0.09 \pm 0.03	0.07 \pm 0.02	0.09 \pm 0.03
NO ₃ ⁻ -N (μ g g ⁻¹ dry soil)	1.62 \pm 0.21	1.40 \pm 0.19	0.93 \pm 0.10	0.90 \pm 0.09

6.2.2 Closed incubation system and incubation of the intact soil cores

The experimental procedure was the same as that described in Chapter 5 (section 5.2.2). The exception was that the upper bag was inflated with a mixture of 125 ml of air and 15 ml of acetylene (giving approximately 10 % C_2H_2 by volume). The total pore volume in the intact cores was calculated as 138 cm^3 , and 20 ml of air was withdrawn using a syringe and replaced by 20 ml of acetylene (approximately 10 % by volume). Gas samples and analyses, infiltration rate and soil strength measurements and soil analysis were conducted as described in sections 5.2.4, 5.2.5 and 5.2.6.

6.2.3 Data analysis

The unbalanced factorial design was used to investigate the factors and their interaction effects on total denitrification rates, infiltration rate and soil strength of the intact topsoil irrigated cores. Data from the experiment was prepared and tabulated in Excel and the means of C-amended treatments were calculated. Denitrification rates were normalized by log-transformation (natural log) prior to statistical analysis (Parkin, 1993). In this unbalanced design, analysis of variance was carried out using the General Linear Model command. Statistical analysis was conducted on mean denitrification/ respiration rates of day 1 and 2 of the experiment. All statistical analyses were carried out using MiniTab 12.

6.3 Results

Denitrification rates and CO_2 production measured in incubations of irrigated soil cores are shown in Figures 6.1 and 6.3.

For all treatments, the highest N_2O fluxes were observed after 2 days' incubation, after which they decreased (Figure 6.1a, b). The exception was replicate number one of the uncrusted soil cores irrigated with wastewater and with added carbon (T2+C) (Figure 6.1a).

Total denitrification rates observed in crusted and uncrusted carbon- amended cores from wastewater- irrigated plots were higher than those observed in the similar cores from well irrigated-plots (Figure 6.1a, b).

Total denitrification rates in C-amended cores from wastewater-irrigated plots (Figure 6.1a) were higher when crusted than when uncrusted, except for replicate number 1 of the uncrusted wastewater-irrigated treatment (T2+C) (Figure 6.1a).

The maximum total denitrification rates observed in crusted and uncrusted C-amended cores irrigated with wastewater were 92.9 and 58.9 $\mu\text{g N}_2\text{O-N kg}^{-1} \text{ day}^{-1}$ respectively, compared with maximum rates of 1.50 and 1.30 $\mu\text{g N}_2\text{O-N kg}^{-1} \text{ day}^{-1}$ observed from the corresponding cores of soil irrigated with well water (Table 6.3).

Carbon dioxide emission rates in soil cores from either wastewater or well water – irrigated plots were quite similar (Figure 6.3a, b, Table 6.3). CO_2 production rates were not associated with the total denitrification rates (Table 6.3). There was a good correlation between the total amount of CO_2 emitted and total $\text{N}_2\text{O-N}$ gas emission in intact cores from well water-irrigated plots ($r^2=0.67$, $p<0.05$) (Figure 6.5).

The mean of the total denitrification rates observed from soil cores irrigated with wastewater under all treatments ranged from 1.08 to 24.2 $\mu\text{g N kg}^{-1} \text{ dry soil day}^{-1}$, while the mean denitrification rates observed from soil cores irrigated with well water ranged from 1.03 to 1.16 $\mu\text{g N kg}^{-1} \text{ dry soil day}^{-1}$ (Table 6.3).

The mean of the total denitrification rates of intact crusted and uncrusted C-amended soil cores from wastewater-irrigated plots (Table 6.3) were much greater than those from unamended soil cores (Table 6.3). For the equivalent soil cores from well water-irrigated plots, the means of the total denitrification rates observed from C-amended and unamended soil cores were nearly the same (Table 6.3).

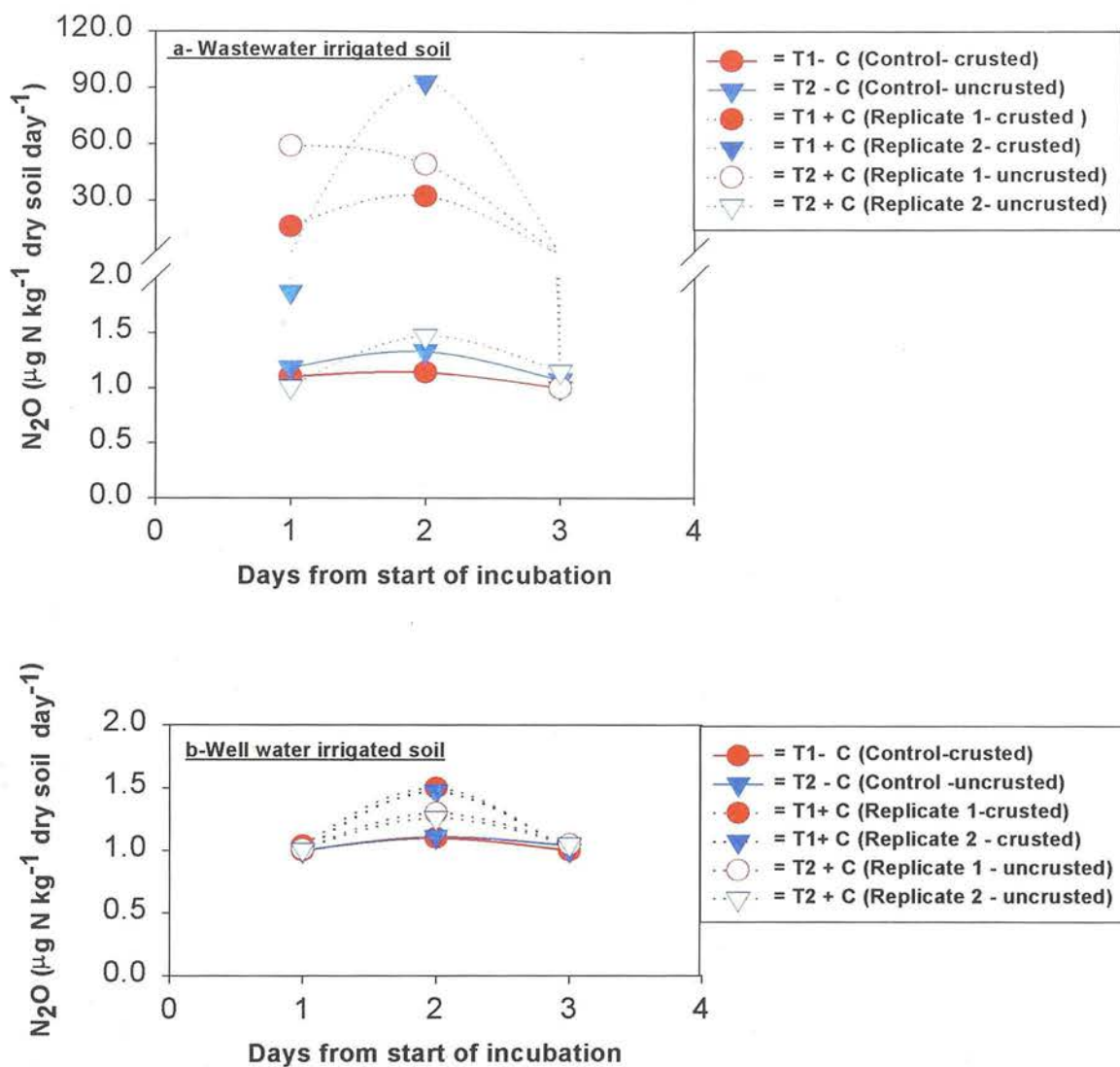


Figure 6.1 Denitrification rates in crusted and uncrusted intact topsoil from (a) wastewater and (b) well water-irrigated plots, with and without C-amendment.

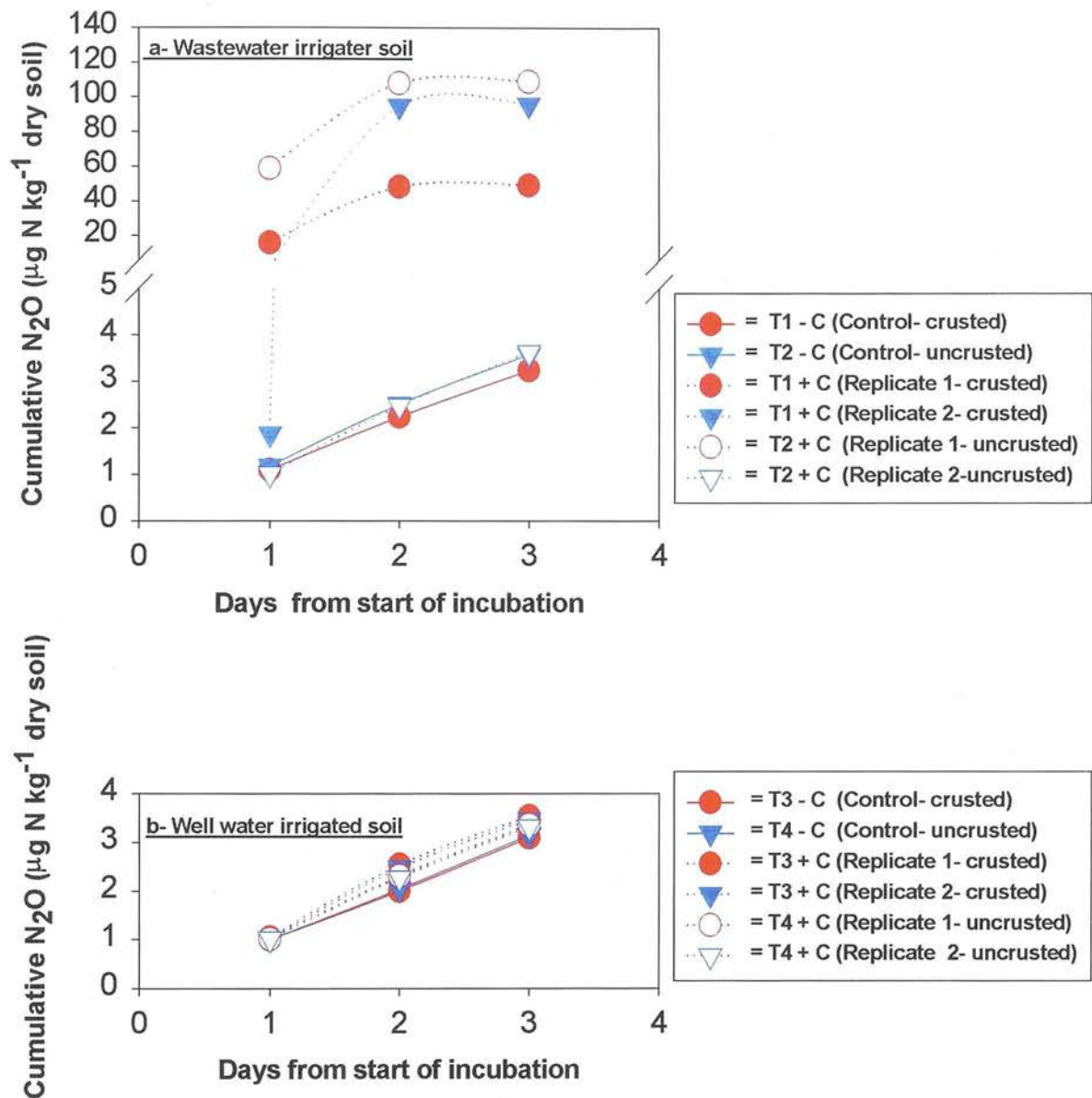


Figure 6.2 Cumulative N_2O emitted from incubations of intact topsoil from (a) wastewater and (b) well water-irrigated plots, with and without C-amendment.

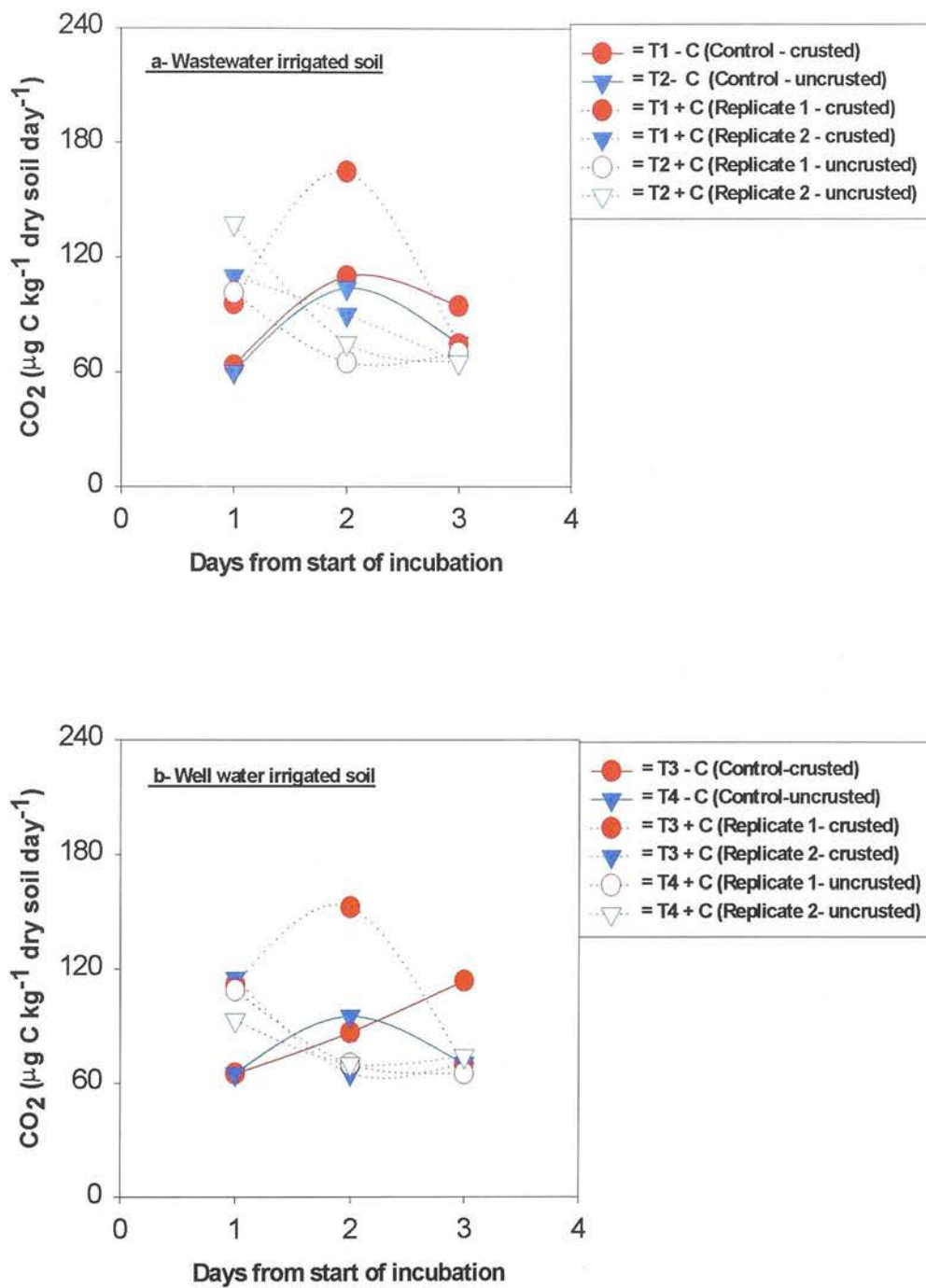


Figure 6.3 Carbon dioxide (CO₂) evolution from incubations of intact topsoil cores from (a) wastewater and (b) well water-irrigated plots, with and without C-amendment.

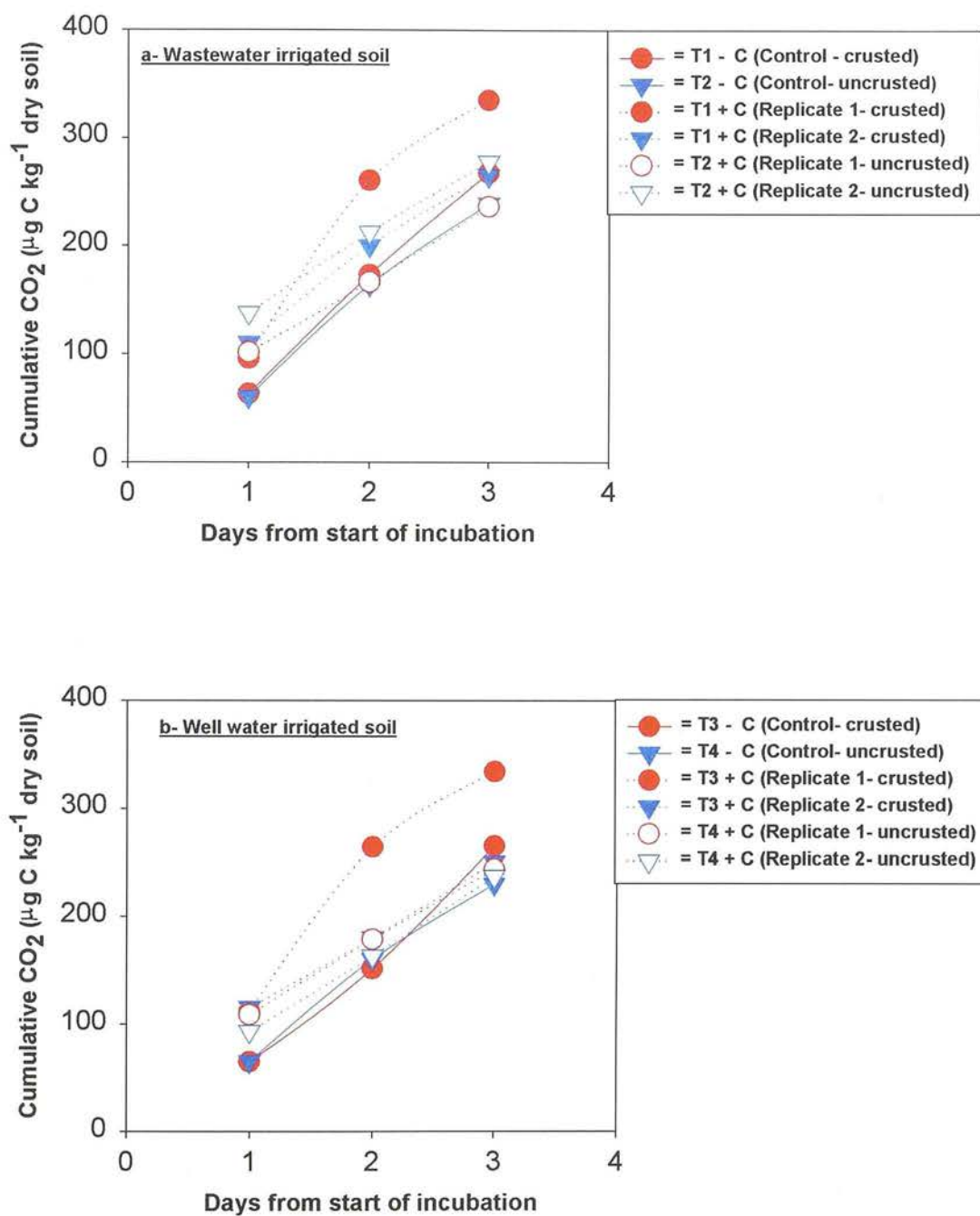


Figure 6.4 Cumulative CO₂ emitted from incubations of intact topsoil cores from (a) wastewater and (b) well water-irrigated plots, with and without C-amendment.

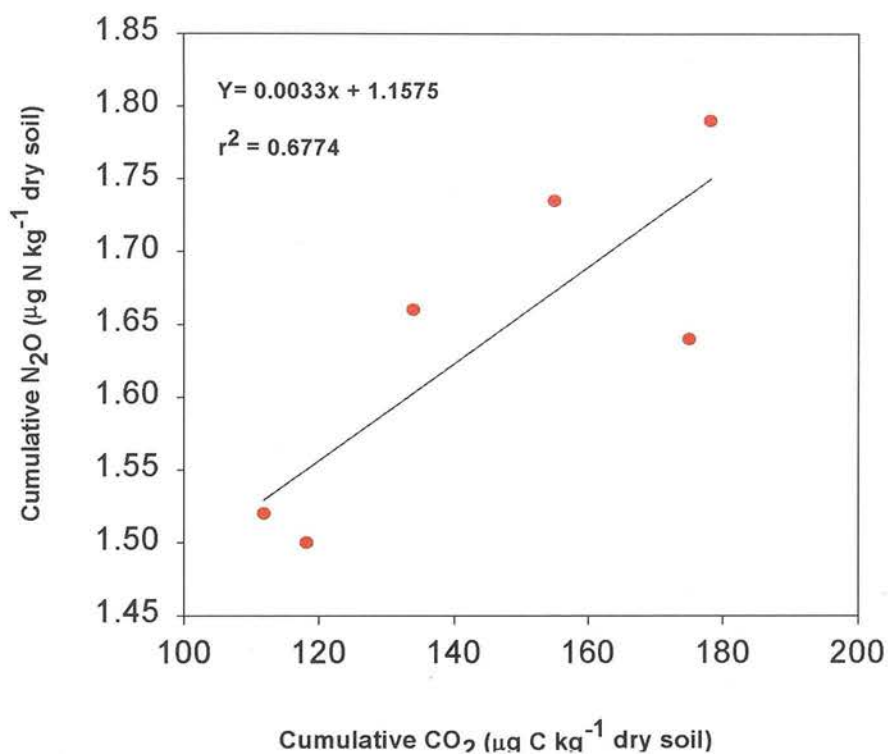


Figure 6.5 Correlation between the mean cumulative CO₂ emissions for day 1 and 2 versus the corresponding cumulative N₂O emitted from incubations of intact topsoil cores of well water- irrigated plots with and without C-amendment.

Table 6.3 Maximum of the individual values and mean rates of days 1 and 2 of the experiment for total denitrification ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day^{-1}) and carbon dioxide production ($\mu\text{g CO}_2\text{-C kg}^{-1}$ dry soil day^{-1}) in crusted and uncrusted amended (+ C) and unamended (- C) soil cores from wastewater (Q1) or well water (Q3) irrigated plots. Figures under C- treatments represent averages (n=2).

		denitrification ($\mu\text{g N kg}^{-1}$ dry soil day^{-1})				CO ₂ production ($\mu\text{g C kg}^{-1}$ dry soil day^{-1})			
Irrigated soil		Crusted		Uncrusted		Crusted		Uncrusted	
quality		Max	Mean	Max	Mean	Max	Mean	Max	Mean
Q1	+C	92.9	35.7	58.9	27.6	164	115	137	94.7
	-C	1.14	1.12	1.33	1.25	110	86.5	103	81.9
Q3	+C	1.50	1.25	1.30	1.14	152	111	108	85.4
	-C	1.10	1.05	1.11	1.05	86.5	75.7	95.0	80.0

Table 6.4 shows the results of the analysis of variance for the main factors and their interactions in influencing total denitrification rates in intact soil cores. These results indicate that the total denitrification rates were significantly ($p<0.05$) affected by the irrigated soil quality in the field (Q1, Q3). However, carbon amendment (+C, -C) and crust formation (crusted, uncrusted) had no significant effect on total denitrification rates measured in intact soil core incubations (Table 6.4).

The effect of the main factors and their interactions on carbon dioxide production rates was not significant (Table 6.5).

Table 6.4 Analysis of variance for different sources of variation and their interactions effect on the mean denitrification rates ($\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day⁻¹) of days 1 and 2 of the experiment in incubated intact soil cores. NS = not significant at $p>0.05$. * = Significant at $p<0.05$.

Source	DF	SS	MS	Significance
Irrigated soil quality (Q1, Q3)	1	3.4201	2.4103	*
Carbon amendment(+C, -C)	1	0.4419	0.4419	NS
Crusted formation(crusted,uncrusted)	1	0.0553	0.0266	NS
Soil quality*carbon	1	0.3286	0.3286	NS
Soil quality*crust formation	1	0.0366	0.0177	NS
carbon*crust formation	1	0.0310	0.0310	NS
Soil quality*Carbon*crust formation	1	0.0204	0.0204	NS
Error	4	0.6732	0.1683	
Total	11	5.0071		

Table 6.5 Analysis of variance for different sources of variation and their interactions effect on the mean of carbon dioxide production ($\mu\text{g CO}_2\text{-C kg}^{-1}$ dry soil day⁻¹) of days 1 and 2 of the experiment in incubated intact soil cores. NS = not significant at $p>0.05$.

Source	DF	SS	MS	Significance
Irrigated soil quality (Q1, Q3)	1	129.2	113.0	NS
Carbon amendment(+C, -C)	1	1124.9	1124.9	NS
Crusted formation(crusted,uncrusted)	1	712.6	359.4	NS
Soil quality*carbon	1	0.1	0.1	NS
Soil quality*crust formation	1	0.2	2.4	NS
carbon*crust formation	1	346.9	346.9	NS
Soil quality*Carbon*crust formation	1	33.3	33.3	NS
Error	4	1635.8	409.0	
Total	11	3983.0		

From the final balance sheet (Table 6.6) which was used to evaluate the accuracy of these measurements, i.e. to quantify whether or not there was more N lost as denitrified gas than there was actually measured from the soil cores as N₂O emission, it was observed that an average of 13.4 and 12.0 % of the nitrogen

fertilizer applied to wastewater and well water irrigated topsoil cores, respectively, was unaccounted for at the end of incubation.

Overall, 82.9 to 89.8 % (mean 86.6 %) of the N fertilizer applied to topsoil cores irrigated with wastewater, and 86.8 to 88.6 % (mean 87.8 %) of the N applied to soil cores irrigated with well water, was recovered at the end of the incubation period (Table 6.6).

Losses of N fertilizer by denitrification as N_2O gas were greatest in topsoil cores irrigated with wastewater, accounting for an average of 1.02 %, compared with 0.07 % under well water irrigation (Table 6.6).

Table 6.6 Nitrogen balance sheet for intact soil cores from wastewater and well water-irrigated plots and incubated in the presence of 10 % (by volume) acetylene.

Irrigated soil quality	Soil irrigated with wastewater						Soil irrigated with well water					
NO ₃ ⁻ added (µg N g ⁻¹)	4						4					
Treatments	T1+C R1	T1+C R2	T1-C	T2+C R1	T2+C R2	T2-C	T3+C R1	T3+C R2	T3-C	T4+C R1	T4+C R2	T4-C
N balance (µg N)	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500
Fertilizer solution												
N ₂ O	17.4	33.8	1.14	38.5	1.28	1.26	1.25	1.22	1.09	1.18	1.17	1.11
Final soil	1254	1211	1337	1264	1289	1346	1315	1319	1329	1317	1301	1325
N unaccounted for	228	255	161	197	209	152	183	179	169	181	197	173
N ₂ O-N %	1.16	2.25	0.07	2.57	0.08	0.08	0.08	0.08	0.07	0.07	0.07	0.07
¹⁵ N % in final soil	83.6	80.7	89.1	84.2	85.9	89.7	87.6	87.9	88.6	87.8	86.7	88.3
N-recovery %	84.7	82.9	89.2	86.8	86.0	89.8	87.7	88.0	88.6	87.8	86.8	88.4
N % unaccounted for	15.2	17.0	10.7	13.1	13.9	10.1	12.2	11.9	11.3	12.1	13.1	11.5

R1= Replicate number 1
R2= Replicate number 2

where

All treatments are as specified in materials and methods (section 6.2.1, Table 6.1)

Infiltration rates (IR) of intact cores under all treatments ranged from 8.0 to 23.5 cm h⁻¹ (Table 6.7), whereas the means of the soil strengths of the same soil cores measured at several spots ranged from 9.9 to 11.6 kg cm⁻² (Table 6.7). The lowest soil infiltration rate of 8 cm h⁻¹ and the highest soil strength of 11.6 kg cm⁻² were observed in the crusted core from wastewater irrigated soil without C-amendment (T1-C). The highest IR of 23.5 cm h⁻¹ and the lowest soil strength of 9.9 kg cm⁻² were measured in one out of the two uncrusted C-amended cores from well water-irrigated plots T4+C (Table 6.7).

Table 6.8 shows results of the analysis of variance for main factors and their interactions in influencing the infiltration rate (IR) of intact topsoil cores with and without C-amendment. The analysis shows that IR at intact soil cores was very significantly affected ($p < 0.001$) by the irrigation water quality (Q1, Q3) and by the crust formation (crusted, uncrusted cores). IR was also highly significantly ($p < 0.01$) affected by carbon amendment (+C, -C). However, there was no significant interaction effect between the main factors on the infiltration rates of the intact soil cores (Table 6.8).

Table 6.7 Infiltration rate (cm h^{-1}) measured once using a single ring infiltrometer of 3.8 cm diameter and 1 cm height, and the means of soil strength (kg cm^{-2}) measured at six points in each intact soil core from either wastewater or well-water irrigated plots under different treatments.

Irrigated soil quality	Treatments	Mean Soil strength rates (kg cm^{-2})	Infiltration rates (cm h^{-1})
Q1	<u>Crusted</u>		
Soil irrigated with wastewater	T1+C	11.4	9.5
	T1-C	11.6	8.0
	<u>Uncrusted</u>		
	T2+C	10.9	16.5
	T2-C	11.2	15.0
Q3	<u>Crusted</u>		
Soil irrigated with well water	T3+C	10.6	15.0
	T3-C	10.9	12.0
	<u>Uncrusted</u>		
	T4+C	9.9	23.5
	T4-C	10.1	21.0

R1 = Replicate number 1

R2 = Replicate number 2

where

All treatments are as specified in materials and methods (section 6.2.1, Table 6.1)

Table 6.8 shows results of the analysis of variance for the main factors and their interactions in influencing infiltration rate (IR) for intact topsoil cores with and without C-amendment. The analysis shows that IR at intact soil cores was very significantly affected ($p<0.001$) by the irrigation water quality (Q1, Q3) and by the crust formation (crusted, uncrusted cores). IR was also highly significantly ($p<0.01$) affected by carbon amendment (+C, -C). However, there was no significant interaction effect between the main factors on the infiltration rates of the intact soil cores (Table 6.8).

Table 6.8 Significant values of main factors and their interaction effects on the infiltration rate (cm h^{-1}) of irrigated soil cores in the presence and absence of carbon amendment. NS = not significant at $p>0.05$; ** = significant at $p<0.01$; *** = significant at $p<0.001$.

Source of variation	DF	SS	MS	Significance
Irrigated soil quality (Q1, Q3)	1	102.083	102.083	***
Carbon amendment (+C, -C)	1	12.042	12.042	**
Crus formation (crusted, uncrusted)	1	184.083	184.083	***
Soil quality*Carbon	1	1.042	1.042	NS
Soil quality*crust formation	1	2.083	2.083	NS
Carbon*crust formation	1	0.042	0.042	NS
Soil quality*Carbon*crust formation	1	0.042	0.042	NS
Error	4	1.500	0.375	
Total	11	302.917		

Table 6.9 shows the results of the analysis of variance for the main factors and their interaction effects on the soil strength of irrigated intact cores with and without C-amendment. This table indicates that the soil strength of irrigated cores was very significantly ($p<0.001$) affected by irrigation quality in the field and crust formation in intact soil cores, and was significantly ($p<0.05$) affected by carbon amendment. In addition, the effect of crust formation was significantly ($p<0.05$) dependent on irrigation quality in the field. However there was no significant interaction effect between crust formation and carbon amendment on the soil strength of intact cores (Table 6.9).

Table 6.9 - Analysis of variance of the main factors and their interaction effects on the soil strength (kg cm^{-2}) of intact soil cores with and without C amendment. NS = not significant at $p>0.05$; * = significant at $p<0.05$; *** = significant at $p<0.001$.

Source of variation	F	SS	MS	Significance
Irrigated soil quality (Q1,Q3)	1	2.52083	2.22042	***
Carbon amendment (+C, -C)	1	0.12042	0.12042	*
Crust formation (crusted, uncrusted)	1	1.14083	1.00042	***
Soil quality*carbon	1	0.00042	0.00042	NS
Soil quality*Crust formation	1	0.06750	0.07042	*
carbon*Crust formation	1	0.00042	0.00042	NS
Soil quality*carbon*Crust formation	1	0.00375	0.00375	NS
Error	4	0.03500	0.00875	
Total	11	3.88917		

6.4 Discussion

C-amended soil cores were expected to exhibit the highest denitrification rates. This is because the available C source can promote denitrification in two ways (Rolston, 1981):

1. Available C acts as an electron donor promoting heterotrophic denitrification.
2. Increased heterotrophic respiration leads to O_2 demand exceeding supply, and subsequently the development of anoxic microsites conducive to denitrification.

In laboratory experiments, addition of degradable organic material in the form of glucose was found to increase N_2O emissions through the denitrification process (Eaton and Patriquin, 1989; Azam *et al.*, 2002).

Previous studies have indicated that denitrification is significantly correlated with water-soluble organic carbon (WSOC) and organic carbon (Burford and Bremner, 1975; Beauchamp *et al.*, 1980). Statistically, the difference in total denitrification rates observed in intact C-amended and unamended cores was not significant. This might be due to the small amount of added carbon amendment ($1.7 \mu\text{g C g}^{-1}$ dry soil added during incubation with nitrate (section 6.2.1). An addition of $1.7 \mu\text{g C g}^{-1}$ dry soil is unlikely to have had an effect. This suggests that more research is needed to

study denitrification under different levels of organic carbon amendments, for example adding a range of different C concentrations, instead of using one concentration level only. The rate of denitrification in soil depends largely on the amount of organic soil C that is readily available to denitrifying micro-organisms (Bremner and Shaw, 1958a; Burford and Bremner, 1975). However, Malhi *et al.* (1990) reported a poor correlation between total organic C and NO_3^- -N loss ($r^2 = 0.34$), but suggested that this was due to factors other than C-limiting denitrifier activity in the soils.

The initial WSOC concentration in the soil cores irrigated either with wastewater or well water averaged 44 and 33 $\mu\text{g C g}^{-1}$ dry soil respectively (Table 6.2). The difference in available C concentration between the wastewater and well water soil cores may have contributed to the significant difference in denitrification rates observed between them, whereas the addition of an extra 1.7 $\mu\text{g C g}^{-1}$ dry soil is unlikely to have had an effect. Beauchamp *et al.* (1980) reported that concentrations of WSOC in excess of 40 to 80 $\mu\text{g C g}^{-1}$ dry soil were necessary before denitrification was observed in soils. And it may be that in these incubations, the cores from wastewater- irrigated plots with 45.7 $\mu\text{g C g}^{-1}$ dry soil, were above the threshold necessary for this process to take place, whereas those with 33 $\mu\text{g C g}^{-1}$ dry soil were below the threshold (cores irrigated with well water). Although, the statistical analysis showed a significant increase in denitrification rate of wastewater-irrigated soil cores compared with well water-irrigated soil cores, the maximum denitrification rate observed from wastewater-irrigated soil cores was not more than 92.9 $\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day^{-1} .

Another possibility for the significant increase in total denitrification rates observed in soil cores from wastewater- irrigated plots from those measured in soil cores from well water-irrigated plots might be that the combined effect of the finer texture, and higher exchangeable sodium percentage (ESP) of soil irrigated with wastewater in the presence of 45.7 $\mu\text{g C g}^{-1}$ dry soil has the potential to give higher N_2O losses (Figure 6.1a). Unfortunately, we can not tell which is responsible; more replications are needed because of the variability of the data.

Due to the many factors that affect denitrification rate and the natural spatial variability in soil, it was not possible to pinpoint the factors that caused the differences in denitrification rates observed in the two replicates irrigated with wastewater. Spatial variability in soil can be due to physical, chemical or biological heterogeneity over a wide range of scales. Soil denitrification typically exhibits high variability and skewed distribution due to the dynamic nature of the micro-organisms involved in the process (Parkin, 1987). A large proportion of denitrification variability within natural soil cores has been reported to occur at distances of <10 cm (Parkin *et al.*, 1987), and up to 50 % of the variance within a field may be present within one square metre (Biggar, 1978).

Total denitrification rates observed in uncrusted C-amended soil core from wastewater-irrigated plots (T2+C, Replicate 1-uncrusted) were higher than those observed in replicate 1 of the corresponding crusted core (T1+C Replicate 1-crusted). Denitrifiers would be expected to remain more active under crusted conditions, and these results suggest that soil structure may not be the main factor limiting denitrification in intact soil cores irrigated with wastewater. This is confirmed by the results from the statistical analysis showing that the crust formation had no significant effect on total denitrification rates in soil core incubations.

The readily available C:N ratios (WSOC: mineral N ratio) can be considered as the most important factor for micro-organisms under the incubation conditions. Unfortunately, topsoil used in these incubations were not analysed for water-soluble organic carbon and mineral nitrogen at the end of incubation. Rates of C: N>20 tend to favour immobilisation (Lynch, 1988). The C: N ratio of added carbon and nitrogen in these incubations is 3.3.

Weier *et al.* (1993a) reported a strong relationship between emissions of CO₂ and N₂O in intact clay topsoil cores from a depth of 5-10 cm receiving NO₃⁻ + glucose and NO₃⁻ alone. In contrast, in our study, rates of CO₂ emission in intact soil core incubations were not associated with denitrification rates. There was a good correlation between total µg CO₂ emitted and total µg N₂O-N gas emission in intact

soil core incubations, irrigated with well water in the field. This suggests that biological rather than chemical denitrification was occurring, and that CO₂ was a good indicator of microbial activity in the intact topsoil cores. Rates of CO₂ emission were of a similar magnitude in wastewater and well water-irrigated soil core incubations, indicating that microbial activity was occurring at a similar rate.

In C-amended soil cores from wastewater- irrigated plots, 0.18 µg N was denitrified per µg C released as CO₂, whereas in unamended soil cores from the same plots, 0.015 µg N was denitrified per µg C released as CO₂. However, in amended and unamended soil cores from well water- irrigated plots, the effective ratios were similar, 0.013 and 0.011 µg N denitrified per µg C released as CO₂ respectively.

Potential denitrification rates of 1.33 and 1.11 µg N₂O-N kg⁻¹ dry soil day⁻¹ were observed from incubations of intact topsoil cores from wastewater and well water-irrigated plots respectively in the absence of any additional C. These rates are lower than those reported in other studies looking at potential denitrification in topsoil irrigated and not irrigated with wastewater, and in the absence of carbon amendment, using the acetylene block method. Studying the denitrification in Mexican topsoil samples (0-15 cm depth) irrigated for 80 years using either fresh or wastewater, Friedel *et al.* (2000) reported potential denitrification rates of 105.6 and 739.2 µg N₂O-N kg⁻¹ dry soil day⁻¹ respectively, without C-amendment. The higher potential denitrification observed in these studies might be due to the presence of high total soil organic carbon content under both irrigation qualities. Another possibility might be that the high potentially mineralizable carbon (162 and 486 µg C g⁻¹ dry soil) in their study was causing higher potential denitrification.

Potential denitrification rates of 92.9 and 1.50 µg N₂O-N kg⁻¹ dry soil day⁻¹ were observed in incubations of intact topsoil cores from wastewater and well water irrigated plots, respectively, in the presence of added carbon. These rates are lower than those reported in other studies. Bremner and Shaw (1958b) reported that the loss of nitrogen applied to a clay loam topsoil incubation, calculated as a percentage of the nitrate-N added, was 71 %. The higher potential denitrification observed in Bremner and Shaw's study might be due to the high C:N ratio of the glucose-nitrate

solution added during incubation was equivalent to 20, whereas in our study, the C:N ratio of the glucose-nitrate solution added during incubation was 0.39.

From the nitrogen balance, the average percentage of nitrogen recovered from soil core incubations from either wastewater or well water-irrigated plots were estimated to be 86.6 and 87.8 % respectively. However, the calculated average percentages of the nitrogen fertilizer applied to these cores that were unaccounted for at the end of incubation, were 13.4 and 12 % respectively. These values may be partly explained by analytical errors or might be because a further reduction of N_2O to N_2 occurred in soil core incubations.

6.5 Overview

Irrigated soil quality in the field was found to be an important factor affecting topsoil denitrification rates in intact soil cores from the Galicos River site. The results presented here suggest that in crusted and uncrusted topsoil cores irrigated either with wastewater or well water without additions of high amounts of C, denitrifiers can not be active and therefore cannot produce significant levels of denitrification which would result in the removal of NO_3^- from the soil before becoming a potential pollutant. It is surprising that in spite of the carbon addition of only $1.7 \mu\text{g C g}^{-1}$ dry soil, it had a highly significant effect on increasing infiltration rate of intact soil cores and had a positive effect on decreasing soil strength. In contrast, crust formation induced on the surface of intact soil cores had no significant effect on total denitrification rates in intact soil cores but it had a highly significant effect on decreasing IR and increasing soil strength. Overall, 82.9 to 89.8 and 86.8 to 88.6 % of the N fertilizer applied to intact soil cores irrigated either with wastewater or well water in the field were recovered at the end of incubation, respectively.

7 Denitrification and soil texture in intact cores of clay soil from the Axios Delta

7.1 Introduction

The economic and environmental concerns associated with the denitrification of N fertilizer from agricultural soils have prompted many studies to determine the fate of N in soil (Rolston *et al.*, 1982; Arah *et al.*, 1991; Vinten *et al.*, 1992).

It is generally considered that denitrification is greater in finer-textured clay soils than in coarse-textured sandy soils (Foster *et al.*, 1986). Greater rates of denitrification have also been reported in finer-textured soils (Lund *et al.*, 1974).

Soil texture influences the denitrification process in several ways. Firstly, exposed soil surfaces provide attachment sites for microbial cells, and fine-textured soils have a greater surface area. Secondly, negatively charged soil colloids may concentrate nutrients in the soil solution (Focht and Verstraete, 1977), and these colloids are more abundant as the texture becomes finer and the clay content increases. Other effects of soil texture on denitrification are likely to result from physical variations in soil structure, pore size, aggregation and water infiltration rates that affect aeration and water holding/absorption capacity.

Soil texture may affect organic matter turnover by adsorption of organic matter onto the surface of clays, to create clay-organic complexes (Oades, 1988). Texture also influences soil structure (Sommers *et al.*, 1981; Schjønning *et al.*, 2003) by modifying the environmental conditions under which decomposition takes place (e.g. pore size distribution, gas exchange, diffusion of substrates).

N₂O production and emission is influenced by soil water content, compaction or localized high aerobic microbial activity (Davidson, 1991; Skiba *et al.*, 1992). Thus, factors that determine the aeration of a soil such as soil texture can significantly influence the rate of N₂O emissions.

Soil type has been reported to have a significant effect on denitrification. Clay soils can have a higher potential for sustained N_2O formation than sandy soils (Granli and Bøckman, 1994). However, reduction of N_2O to N_2 is favoured in soils with very high clay content, and such soils can show low N_2O emission rates compared to coarser soils (Arah *et al.*, 1991). This soil texture effect is associated with other factors, especially soil air and water content. Burton and Beauchamp (1985) found that an air-filled porosity in excess of 35 % allowed sufficient supply of O_2 to inhibit denitrification in cores of silty loam topsoil.

The objective of the present study was to investigate the differences in N denitrification losses from different textured soils in the Axios Delta fields.

7.2 Materials and Methods

7.2.1 Field site

The Axios Delta is a RAMSAR (The Convention on Wetlands, 1971) site of international importance for the value of the wetland habitat. Several international organizations have recognized the ecological significance of the Axios Delta and its catchment and it has been listed as one of the most important ornithological shelters in Europe protected by the Ramsar Convention. In addition to these natural habitat types there is a matrix of rice fields on the delta.

The Axios Delta fields (Map 7.1) from where the intact topsoil cores were collected form part of the Axios alluvial plain. This is one of the most productive areas of Greece and is formed by the alluvial deposits and deltas of the rivers Axios, Gallikos and Ludias and their tributaries. There is a complicated pattern of land use, mainly the growing of wheat, barley, cotton, maize, rice and vegetables (Silleos, 1988). Field Nos. 2, 3, 4, 5 and 6 had been in continuous arable cultivation for many years up to 2000, with rice and barley as the main crops, while field No.1 was uncultivated. The soil in field No.1 is a saline-alkaline soil with a fluctuating water table.

Sheet erosion, slope, the height of the water table, permeability, concentration of salts, and alkalinity are the main factors which control the productivity and land use in the Axios alluvial plain (Silleos, 1988). The soils are classified at the great group level in the USDA Soil Taxonomy (Soil Survey Staff, 1998) as Xerofluvents.

The catchment of the Axios River covers an area of approximately 25000 km². The western and southwestern part of the catchment is mountainous and the highest altitude is about 2400 m, whereas at the eastern boundary altitudes reach 1800 m. The length of the Axios River in Greek territory is 80 km and its total length is 320 km; it flows through Kilgis and Thessaloniki counties and discharges into the Thermaikos gulf in northern Greece (Eleftheria *et al.*, 2000). It is Greece's second longest river. High discharges of the Axios River occur in springtime due to snow melt from the mountainous areas. The mean annual water discharge is $5 \times 10^9 \text{ m}^3 \text{ y}^{-1}$ and the majority of environmental problems that affect the Axios River catchment arise from human activities.

The climate at the site is as described in Chapter 4 (Section 4.2.1). Climatic data was obtained from Sindos climatological station, which is near the Axios delta and Galicos River sites, for the period from January 1998 to October 2000 (see Map 3.1).

7.2.2 Soil sampling and analysis

Twelve intact topsoil cores were collected from six fields in the Axios Delta (Map 7.1), irrigated by flooding, in April 2000. Two soil cores were taken from each field. Metal rings were hammered into the soil to a depth of 5 cm from the soil surface. Excess soil was removed from the bottom end of the ring. Each core had a height of 5 cm and a diameter of 7.3 cm and contained 272 g dry soil. The bottom and the top ends of each core were both sealed by a plastic lid to protect the soil core during transportation and to prevent evaporation, then each core was sealed in a polythene bag and stored at 4 °C prior to analysis and incubation. Additional soil cores were collected to the same depth and analyzed using methods described in Chapter 3 before incubation for soil electrical conductivity (EC), pH, CaCO₃ %, and

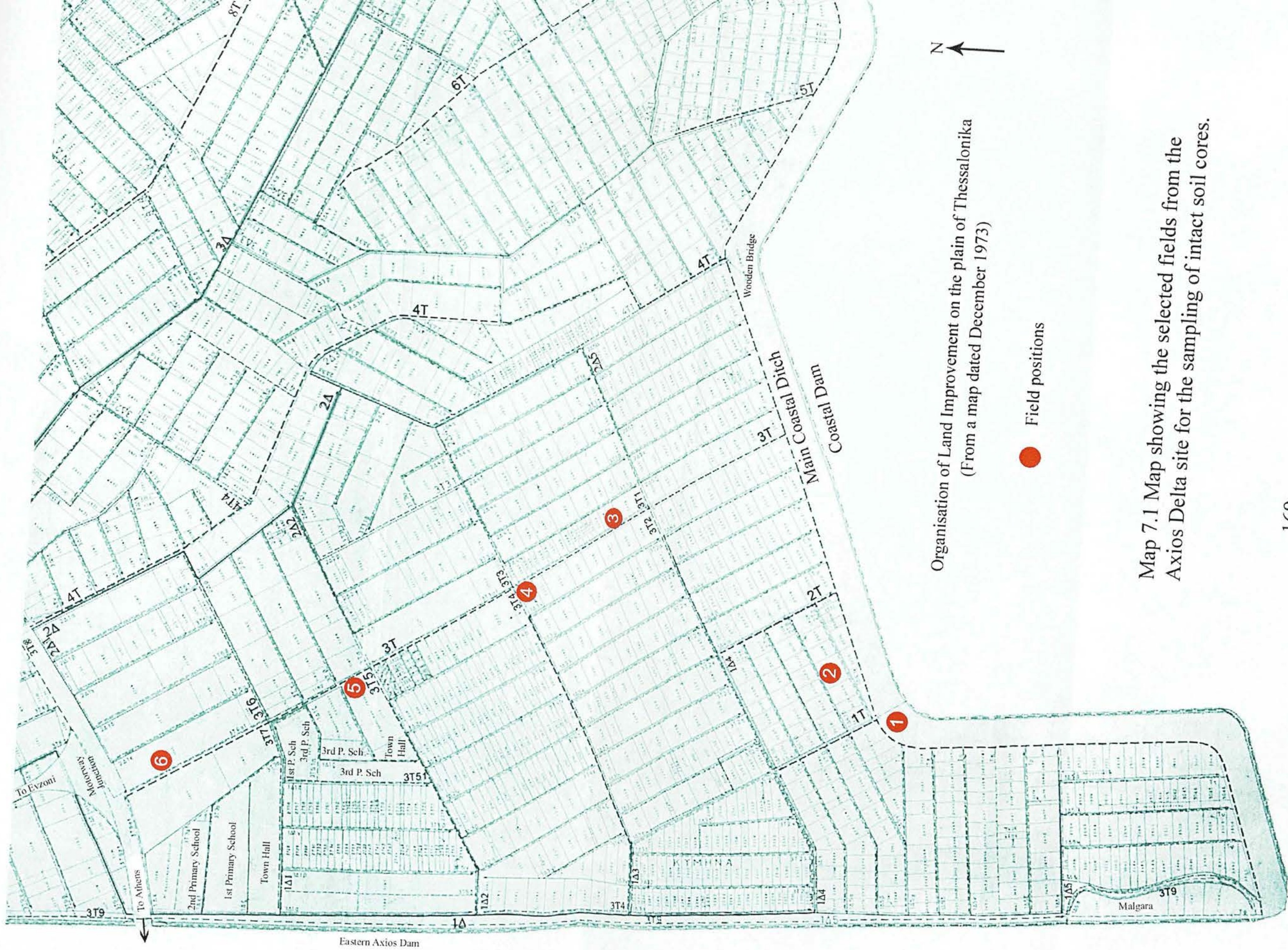
exchangeable sodium and cation exchange capacity, and particle size distribution. Details of the topsoil properties are given in Table 7.1 and Table 7.2.

Table 7.1 Chemical properties of the Axios Delta soil before incubation. Figures represent averages \pm s.d (n=3)

Field. number	CaCO ₃ %	pH	EC (dS m ⁻¹) at 25 °C	ESP %	Soil Salinity Classification
1	6.0 \pm 1.52	7.87 \pm 0.20	6.90 \pm 0.30	16.8 \pm 1.10	Saline-alkali soil
2	5.2 \pm 0.76	7.96 \pm 0.11	0.48 \pm 0.03	2.05 \pm 0.05	Non-saline, non-alkali soil
3	5.0 \pm 0.89	8.11 \pm 0.20	0.40 \pm 0.01	2.52 \pm 0.45	Non-saline, non-alkali soil
4	4.5 \pm 0.17	7.92 \pm 0.05	0.38 \pm 0.01	1.68 \pm 0.17	Non-saline, non-alkali soil
5	4.5 \pm 0.41	8.01 \pm 0.26	0.62 \pm 0.05	2.36 \pm 0.26	Non-saline, non-alkali soil
6	5.0 \pm 0.20	7.88 \pm 0.11	0.65 \pm 0.03	2.38 \pm 0.30	Non-saline, non-alkali soil

Table 7.2 Particle size distribution of the Axios Delta soil.

Field.No	Sand-%	Silt-%	Clay-%	Soil texture
1	6.40	34.88	58.72	Clay
2	6.22	38.96	54.82	Clay
3	0.82	23.81	75.37	Clay
4	4.99	27.99	67.02	Clay
5	39.82	27.33	32.85	Clay loam
6	62.55	20.04	17.41	Sandy loam



Organisation of Land Improvement on the plain of Thessalonika
(From a map dated December 1973)

● Field positions

Map 7.1 Map showing the selected fields from the
Axios Delta site for the sampling of intact soil cores.

Soil cores were saturated with tap water to prevent dispersion of soil aggregates, and placed on a tension table apparatus to drain to field capacity (-5 kPa). Prior to saturation, fine-gauge nylon mesh fabric was placed over the bottom of the intact soil cores and secured with super glue to prevent soil from falling out during the saturation and incubation period.

A 10 ml volume of ^{15}N -labelled nitrogen fertilizer solution (150 mg N l^{-1}), prepared from calcium nitrate containing 99.67 atoms percent of ^{15}N , was added to each soil core. These additions were equivalent to $5.5 \text{ } \mu\text{g N g}^{-1}$ dry soil. The fertilizer solution was prepared using acetylated distilled water as described in section 5.2.1 to carry acetylene more efficiently into the soil pores with the nitrogen solution (Bragan *et al.*, 1997).

Experiments already conducted on the effect of the soil water potential on denitrification and respiration rates in intact soil cores from the Axios Delta site can be seen in Appendix 4.

7.2.3 Closed incubation system and incubation of the intact soil cores

Tedlar bags were fixed to both ends of the incubation system (Figure 5.1). Using a syringe, the upper bag was inflated with a mixture of 96 ml of air and 11 ml of acetylene, giving approximately 10 % C_2H_2 by volume. The total pore volume in the intact cores was calculated as 106.46 cm^3 , using the total soil porosity and the volume of the dry soil in the intact cores. The air-acetylene mixture in the upper filled bag was intended to replace the air displaced by the percolating fertilizer solution. The lower bag was left empty and open to equilibrate the air moving in and out during the percolation of nitrogen fertilizer solution through the soil core. From the head space, 21 ml of air was withdrawn using a syringe and replaced by 21 ml of acetylene, approximately 10 % (by volume), then the system was placed in an incubator at a temperature of $15 \text{ }^\circ\text{C}$ throughout the experimental period (with the exception of sampling times - a few minutes for each sampling).

7.2.4 Gas and soil analysis

A gas chromatograph (GC) fitted with an electron capture detector (ECD) and thermal conductivity detector (TCD) was used to determine the concentrations of nitrous oxide (N_2O) and carbon dioxide (CO_2) respectively. The closed incubation system was linked to the GC using a metal tube, at each sampling time (Plate 5.1). The first gas sample was taken out from the head space immediately and analyzed for CO_2 and N_2O . Then, gas sampling for N_2O and CO_2 analysis was carried out over a 28 day period of incubation. Sampling days are shown in Table 7.3. Following the 28 days' incubation, soil was removed from the metal ring, except for the soil cores from field No.4, which were selected for further denitrification studies. Field No.4 was randomly selected from 4 fields that had soils with a high clay content (Table 7.2), because finer textured soils (high clay content) are considered to have higher denitrification rates than coarse textured soils (low clay content). The depth to which fertilizer solution penetrated inside the soil cores was not measured, but at the end of the incubation time, there was no fertilizer above the surface of the soil cores and none had drained from the soil cores. Since there was no solution drainage, all N_2O emitted was measured in the head space. The soil was mixed well and left to air dry at room temperature for three days. The soil was then sieved (2 mm-mesh size), subsampled and analyzed for water-soluble organic carbon and total carbon contents (Table 7.4). Total carbon content was determined using an automatic carbon analyzer (ACA) in the Land Management Department at the Scottish Agricultural College (SAC) Aberdeen.

Forty days after fertilizer and acetylene addition, and following gas sampling, soil cores from field No.4 were partially dried by circulating cold air in an oven for 3 hours. This was to increase their air filled pore space (AFPS). Then 10 ml of a solution containing 200 mg C l^{-1} was added to each core. These amendments were equivalent to $7.3 \text{ } \mu\text{g C g}^{-1}$ dry soil. Carbon solution was prepared using acetylated distilled water as described in section 5.2.1 to carry acetylene more efficiently into the soil pores with the carbon solution. The incubation system was closed as described in section 7.2.3. From the head space, 21 ml of air was withdrawn using a syringe and it was replaced by 21 ml of acetylene, approximately 10 % (by

volume), then the system was placed in an incubator at a temperature of 15 °C for seven days (with the exception of sampling times).

Gas in the head space was sampled for N₂O and CO₂ analysis. The first gas sample was taken out from the head space immediately and analyzed for carbon dioxide (CO₂) and nitrous oxide (N₂O). Then, gas sampling was carried out over 7 days, to investigate the effect of readily available carbon on the N₂O-N losses from denitrification in these soil core incubations. Sampling days are shown in Table 7.3. Following incubation, soil was removed from the metal ring, mixed well and analyzed for water-soluble organic carbon and total carbon contents.

Table 7.3 Gas sampling times during (a) An incubation experiment using C-unamended soil cores from fields 1-6 and (b) An incubation experiment using C-amended soil cores from field No.4.

Experiment	Sampling days															
	0	1	2	3	4	5	6	7	8	9	10	13	16	22	28	
(a)	x	x	x	x	x			x	x	x	x	x	x	x	x	
(b)	x	x	x	x			x	x								

7.3 Results

Clay soil texture was observed in field Nos. 1, 2, 3 and 4, while in field No. 5 the soil had a clay loam texture. A sandy loam soil texture was observed in field No.6 (Table 7.2). The soil in field No.1 (uncultivated) is saline-alkaline and had higher total carbon and water-soluble organic carbon concentrations than the soils from the other fields (Table 7.1, Table 7.4).

Table 7.4 Total carbon percentage and water-soluble organic carbon concentrations of the Axios Delta soil at the end of incubation time. Values represent the averages (n=2).

Field number	Total carbon %	Water-soluble organic carbon ($\mu\text{g C g}^{-1}$ dry soil)
1	2.91	86.92
2	1.83	17.97
3	1.88	16.65
4	1.99	30.64
5	1.23	13.55
6	1.14	17.00

The maximum and minimum denitrification rates measured from topsoil cores of the Axios delta fields in the presence of natural carbon concentration at field capacity soil moisture status were 178 and 0.71 $\mu\text{g N kg}^{-1}$ dry soil day^{-1} respectively.

Figure 7.1 represents the concentrations of nitrous oxide (N_2O), as measured by electron capture-gas chromatography during incubations of intact topsoil cores from the Axios Delta fields. N_2O results are displayed as $\mu\text{l l}^{-1}$ to monitor the pattern of the N_2O concentration in the head space over the incubation time. This figure shows that, generally, nitrous oxide concentrations initially increased steadily with time for a few days, after which they increased at a much slower rate, and then decreased, when net N_2O production had changed into net consumption. N_2O concentration measured in cores from field Nos. 2, 4 and 5 increased until day 6 (Figure 7.1b, d and e), while in soil cores from field No. 1 it only increased until day 3 of incubation (Figure 7.1a). In cores from field No. 3, N_2O fluxes increased until day 7 (Figure 7.1c). However, N_2O fluxes measured in cores from field No.6 increased until 13 days after incubation (Figure 7.1f).

By far the highest N_2O concentrations were observed in cores from field No. 1 (Figure 7.1a). In cores from field No. 6 (Figure 7.1f), N_2O concentrations were higher than those from field Nos. 2, 3, 4 and 5 (Figure 7.1b, c, d and e) but were still lower than those in the cores from field No. 1 (Figure 7.1a).

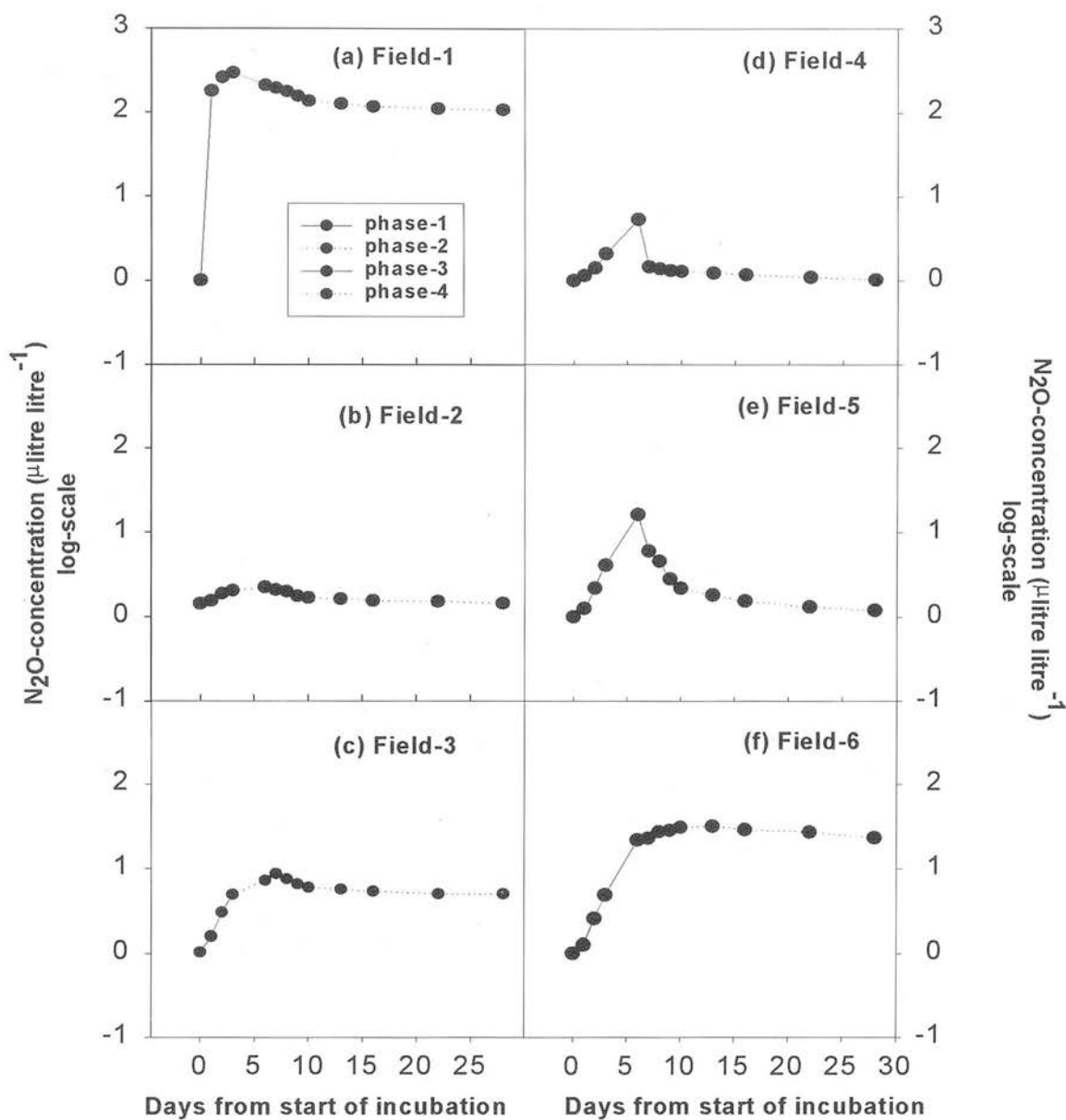


Figure 7.1 Nitrous oxide (N₂O) concentrations, as measured by electron capture-gas-chromatography in incubations of intact topsoil cores from Axios Delta fields over four time periods (phase 1, phase 2, phase 3 and phase 4) at (a) field No. 1, (b) field No. 2, (c) field No. 3, (d) field No. 4, (e) field No. 5 and (f) field No. 6. Each point represents the mean of two replicates (cores).

The trends in N₂O concentrations in all cores can be divided into two periods, an increasing and a decreasing one. Each period can be separated in two phases, giving four phases in all: phase 1 during which N₂O concentrations increased sharply with time following acetylene addition at the beginning of the incubation; phase 2 during which N₂O concentration increased more slowly; phase 3 during which N₂O production had changed into net consumption and N₂O concentrations declined steeply; and phase 4 during which N₂O concentrations continued to decline at a very slow rate, until the end of the incubation period. The average N₂O emissions were calculated up to the point where there was an absolute decrease in the concentrations of N₂O present (i.e. up to the end of phase 2). In order to complement the information obtained about the existence of the separate phases, it was decided to apply simple regression analysis to the data corresponding to the period of increasing and decreasing N₂O concentrations. This allowed for a comparative analysis of N₂O production/consumption to be made easily. Both periods showed linear relationships between the log values of the N₂O concentrations and times of incubation (Figure 7.2a, b). All the equations for the increasing and decreasing periods had high R² values: greater than 67 % and 71 % respectively (Table 7.5). The means of N₂O concentrations measured in the cores, in the presence of natural carbon concentration at field capacity moisture status, ranged from 1.78 to 60.60 µl l⁻¹. The mean concentration was highest in cores from field No. 1 (60.60 µl l⁻¹), followed by field No. 6 (9.52 µl l⁻¹), field No. 3 (3.38 µl l⁻¹), field No. 5 (2.83 µl l⁻¹), field No. 2 (1.80 µl l⁻¹) and with the lowest being in cores from Field No. 4 (1.78 µl l⁻¹) (Table 7.6).

The means of the initial denitrification rate measured in the same cores over phase 1 time period ranged from 0.60 to 81.9 µg N₂O kg⁻¹ dry soil day⁻¹ (Table 7.7). The mean denitrification rate was highest in cores from field No. 1 (81.9 µg N₂O kg dry soil day⁻¹) followed by field No. 6 (2.20 µg N₂O kg dry soil day⁻¹), field No. 3 (1.59 µg N₂O kg dry soil day⁻¹), field No. 5 (1.44 µg N₂O kg dry soil day⁻¹), field No. 4 (0.78 µg N₂O kg dry soil day⁻¹) with the lowest being in cores from field No. 2 (0.60 µg N₂O kg dry soil day⁻¹) (Table 7.7).

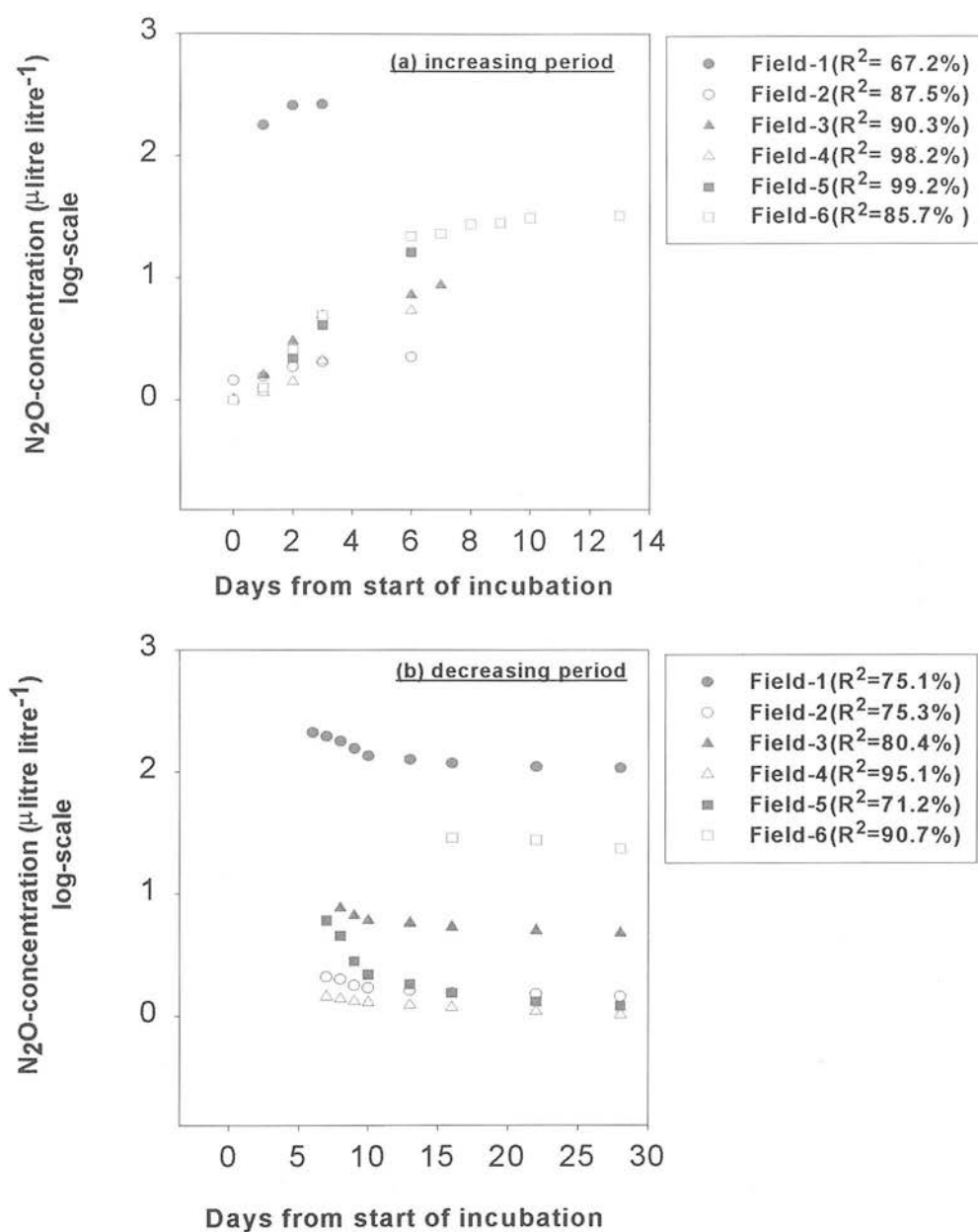


Figure 7.2 Relationship between nitrous oxide concentrations (log-transformation) and time of incubation (day) during (a) increasing period and (b) decreasing period, in soil core incubations of the Axios Delta fields.

Table 7.5 Relationship between log N₂O-N concentrations (C, µl l⁻¹) and time of incubation (T) in soil core incubations of the Axios Delta fields.

Field number	Increasing period		Decreasing period	
	Regression equation	R ² (%)	Regression equation	R ² (%)
1	C = 0.647 + 0.757T	67.2	C = 2.32 - 0.0126T	75.1
2	C = 0.178 + 0.0325T	87.5	C = 0.324 - 0.00663T	75.3
3	C = 0.130 + 0.126T	90.3	C = 0.891- 0.00839T	80.4
4	C = - 0.0509 + 0.126T	98.2	C = 0.186 - 0.00661T	95.1
5	C = - 0.0513 + 0.210T	99.2	C = 0.764 - 0.0286T	71.2
6	C = 0.196 + 0.133T	85.7	C =1.59 - 0.00750T	90.7

Table 7.6 Means of nitrous oxide (N₂O) concentration (µl l⁻¹) as measured by electron capture gas-chromatography in incubations of intact topsoil cores from the Axios Delta fields during the increasing time period (phase 1 + phase 2).

Field-No	Mean concentration (µl N ₂ O-N l ⁻¹)
1	60.6
2	1.80
3	3.38
4	1.78
5	2.83
6	9.52

Table 7.7 Means of initial denitrification rates observed in incubations of intact topsoil cores from the Axios Delta fields during the phase 1 time period. Means that have the same letter after them are not significantly different at $p < 0.001$.

Field-No	Mean initial denitrification rate ($\mu\text{g N}_2\text{O kg}^{-1} \text{ dry soil day}^{-1}$)
1	81.9 ^A
2	0.60 ^B
3	1.59 ^B
4	0.78 ^B
5	1.44 ^B
6	2.20 ^B

A 1-way analysis of variance was carried out to evaluate the differences in initial denitrification rates detected in cores from fields 1, 2, 3, 4, 5 and 6 over the phase 1 time period, and the significantly different pairs of means were identified using Tukey's method (Mead *et al.*, 2003, Ryan and Joines, 2001).

Table 7.8 shows the results of the analysis of variance for source of variation (origin of soil cores i.e. fields) on means of initial denitrification rate measured in cores from fields Nos. 1-6. The mean of the initial denitrification measured in cores from field No.1 was very significantly ($p < 0.001$) higher than the means of denitrification measured in soil cores from fields 2-6. However, there was no significant difference between the means of initial denitrification rates in cores from fields 2, 3, 4, 5 and 6 (Table 7.7).

Table 7.8 Analysis of variance on mean initial denitrification rates ($\mu\text{ g N kg dry soil day}^{-1}$) measured in cores from fields 1, 2, 3, 4, 5 and 6. *** = significant at $p < 0.001$.

Source of variation	DF	SS	MS	F	Significance
Fields	5	3.637	0.727	5.13	***
Error	44	6.239	0.142		
Total	49	9.876			

Concentrations of CO_2 in the cores (Figure 7.3 a-f) increased with incubation time. Concentrations in all cores from fields 2-6 reached a high concentration of 900-2000 $\mu\text{l l}^{-1}$, but those in cores from field No.1 reached 6000 $\mu\text{l l}^{-1}$. Figure 7.3 a-f shows that CO_2 concentrations increased sharply with time following the onset of incubation and acetylene addition (stage A), after which CO_2 concentrations increased at a slower rate which continued until the end of the incubation period (stage B).

The means of the initial respiration rate measured for stage A in soil cores from fields 1-6 ranged from 65 to 467 $\mu\text{g C kg}^{-1}\text{ dry soil day}^{-1}$ (Table 7.9). The mean respiration rate was highest in cores from field No. 1 (467 $\mu\text{g C kg dry soil day}^{-1}$) followed by field No. 2 (153 $\mu\text{g C kg dry soil day}^{-1}$), field No. 4 (133 $\mu\text{g C kg dry soil day}^{-1}$), field No. 3 (130 $\mu\text{g C kg dry soil day}^{-1}$), field No. 5 (67 $\mu\text{g C kg dry soil day}^{-1}$) and the lowest was in cores from Field No. 6 (65 $\mu\text{g C kg dry soil day}^{-1}$) (Table 7.9).

A 1-way analysis of variance was carried out to evaluate the differences between initial respiration rates detected in cores from field Nos. 1, 2, 3, 4, 5 and 6 for stage A. The significantly different pairs of means were identified using Tukey's method (Mead *et al.*, 2003, Ryan and Joines, 2001).

Table 7.10 shows the results of the analysis of variance for source of variation (origin of soil cores i.e. fields) on means of initial respiration rates for stage A measured in cores from field Nos.1-6. These results indicate that the means of the initial respiration rates between fields were very significantly ($p < 0.001$) different.

The mean of the initial respiration rate measured in cores from field No.1 was very significantly higher than the means of respiration measured in soil cores from fields 2-6. However, there was no significant difference between the means of initial respiration rate in cores from fields Nos 2, 3, 4, 5 and 6.

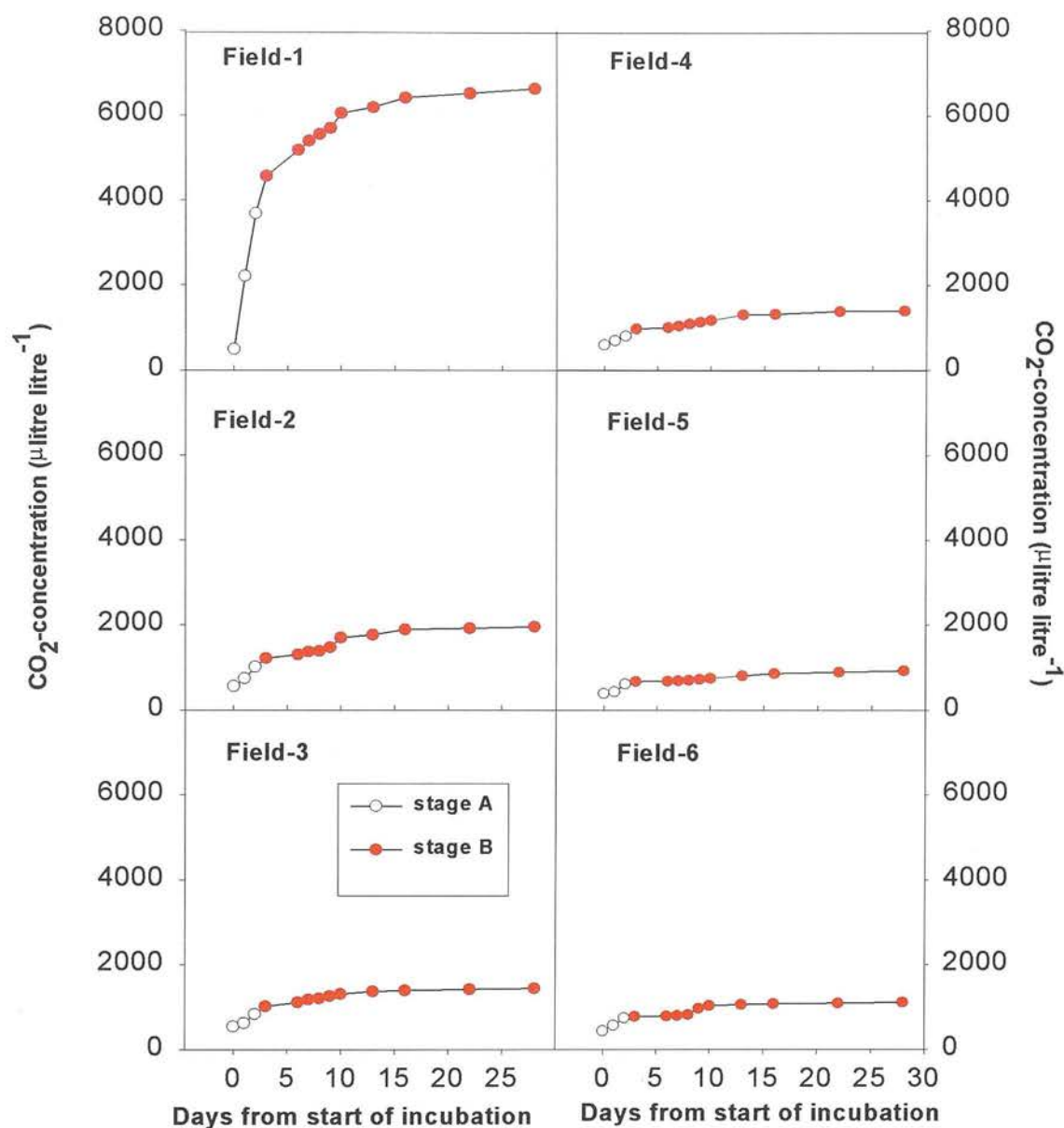


Figure 7.3 Carbon dioxide (CO₂) concentrations as measured by conductivity-gas chromatography in soil core incubations from the Axios Delta fields during incubation time over two stages (stage A, stage B). Each point represents the mean of two replicates (cores).

Table 7.9 Means of initial respiration rate observed in incubations of intact topsoil cores from the Axios Delta fields for stage A. Means that have the same letter after them are not significantly different at $p < 0.001$.

Field-No	Mean initial respiration rate ($\mu\text{g C kg}^{-1} \text{ dry soil day}^{-1}$)
1	467 ^A
2	153 ^B
3	130 ^B
4	133 ^B
5	67 ^B
6	65 ^B

Table 7.10 Analysis of variance on mean initial respiration rates ($\mu\text{g C kg}^{-1} \text{ dry soil day}^{-1}$) measured in cores from fields 1, 2, 3, 4, 5 and 6. * * * = Significant at $p < 0.001$.

Source of variation	DF	SS	MS	F	Significance
Fields	5	906379	181276	12.60	***
Error	42	604031	14382		
Total	47	1510410			

Figure 7.4 shows the daily change in N_2O and CO_2 concentrations measured from topsoil core incubations of field No.4 before and after the addition of carbon amendment. The significance of the difference in total denitrification rates and the in respiration rates of these “Repeated Measures” in intact soil cores from field No.4 before and after the addition of carbon amendment was tested using a two way analysis of variance (Tables 7.11, 7.12).

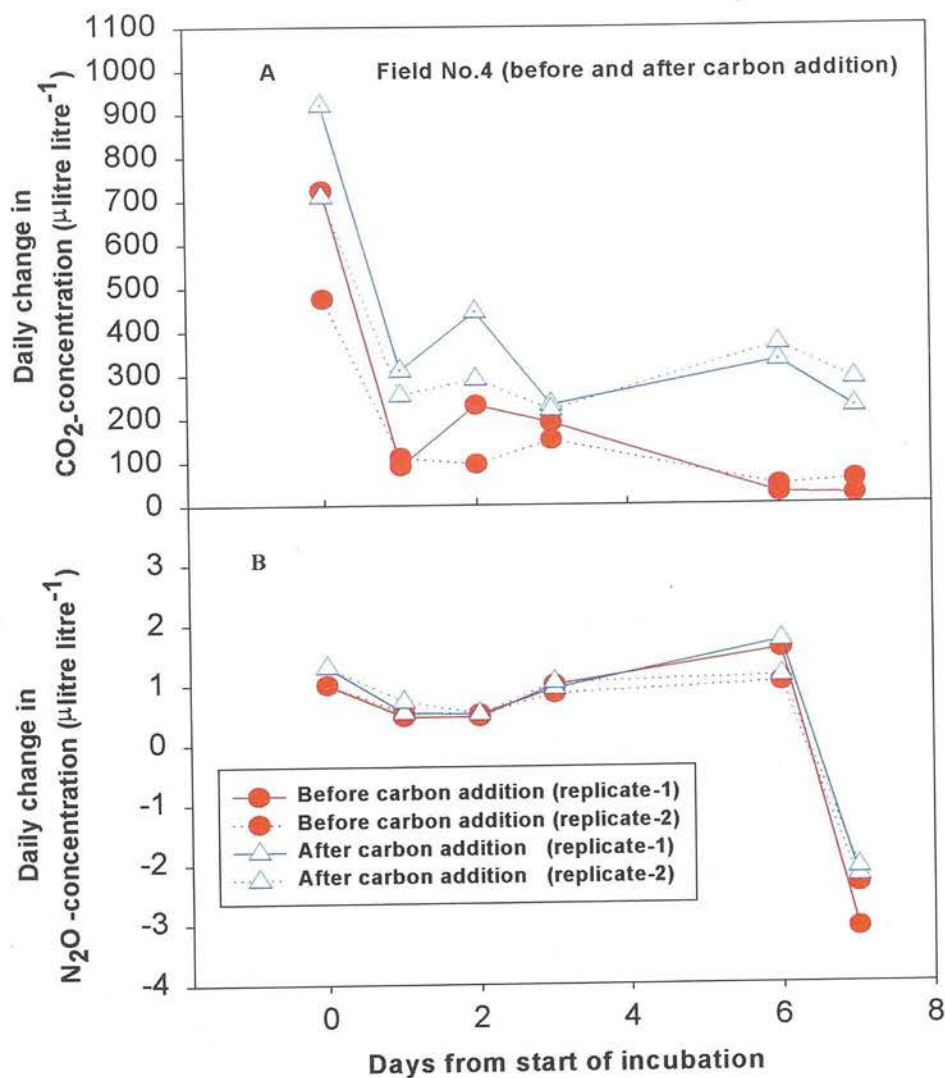


Figure 7.4 Daily change in (a) carbon dioxide and (b) Nitrous oxide concentrations, as measured by electron capture gas-chromatography for soil cores from field No. 4, incubated in the presence of natural carbon concentrations (filled symbols) and with the addition of carbon amendment (open symbols).

Tables 7.11, 7.12 indicate that the difference in total denitrification rates measured in the cores from field No.4 before and after carbon amendment addition was not significant. However, the respiration rates measured in the same soil core incubations after carbon addition were very significantly higher ($p < 0.001$) than rates measured before added carbon. In addition, the effect of incubation time on the mean respiration rate in soil core incubations was very significant ($p < 0.001$). However, incubation time had no significant effect on denitrification rates measured in the same soil cores (Tables 7.11, 7.12).

Table 7.11 Analysis of variance of main factors (Carbon amendment, Incubation time) on total denitrification rate ($\mu\text{g N kg}^{-1}\text{ dry soil day}^{-1}$) in intact soil cores from field No. 4. NS = not significant at $p > 0.05$.

Source of variation	DF	SS	MS	Significance
Carbon amendment	1	0.0002	0.0002	NS
Incubation time	5	0.1073	0.0215	NS
Error	5	0.0507	0.0101	
Total	11	0.1583		

Table 7.12 Analysis of variance of main factors (Carbon amendment, Incubation time) on respiration rate ($\mu\text{g C kg}^{-1}\text{ dry soil day}^{-1}$) in intact soil cores from field.No.4. *** = significant at $p < 0.001$.

Source of variation	D F	SS	MS	Significance
Carbon amendment	1	26782	26782	***
Incubation time	5	157248	31450	***
Error	5	2532	506	
Total	11	186562		

7.4 Discussion

Greater topsoil denitrification rates were expected in the finer textured soil cores compared to the coarser soil cores of the Axios Delta site, due to the smaller particle size of the former, resulting in smaller pores which were more likely to become anoxic zones with increasing moisture and /or microbial CO₂ respiration. Soils from field Nos. 1, 2, 3, 4 were found to have a clay soil texture whereas field Nos. 5 and 6 had a clay loam and sandy loam soil texture respectively.

N₂O production increased exponentially and sharply with increase in time of incubation. This might be attributed to the growth of anaerobic zones over time because of a decrease in oxygen diffusion into soil aggregates and denitrification sites. Previous studies have reported that denitrification rates in soil cores incubated at decreased oxygen concentrations were significantly higher than those incubated in atmospheric O₂ (Arah *et al.*, 1991). They concluded that cores incubated in a static atmosphere are more likely to develop anaerobic microsites (even in a comparatively well-aerated matrix) than cores incubated in a continuous flow system. O₂ concentration in the incubation vessel had a major impact on the denitrification rate of the incubated cores, and the rate increased sharply as external O₂ concentration decreased (Arah *et al.*, 1991). Denitrification rates in soil cores approached anaerobic rates when O₂ concentration fell below 0.5% (Parkin and Tiedje, 1984). Smith (1980) combined models of radial diffusion into aggregates with information on aggregate size distribution in order to estimate the extent of anoxic conditions within the soil profile, and used this information to calculate rates of denitrification. It was assumed that aggregates were spherical in shape, and that there were separate diffusion coefficients for diffusion through the soil profile and radial diffusion through aggregates. Aggregate size distribution, porosity, diffusion coefficients, O₂ uptake rate and NO₃⁻ content were constant down the profile, and the rate of denitrification was dependent only on the fraction of soil that was anoxic. Parkin and Tiedje (1984) reported a good fit of experimental data on denitrification against O₂ concentration when compared with results predicted by the Smith (1980)

model of anoxic volume in soil aggregates, indicating that while this does not confirm the parameters selected for the model, it does suggest that net O₂ diffusion into aggregates or denitrifying sites may be a major factor controlling soil denitrification. At 20 % oxygen concentration, less than 5 % of the potential activity of the denitrifying enzymes was expressed. However, denitrification rate increased as the oxygen concentration was decreased to below 2 % (Tiedje *et al.*, 1984).

The highest denitrification rates were measured in cores from field No. 1, which was uncultivated and had a saline and alkali clay soil texture (Table 7.1, Table 7.2, Table 7.7). This might be due to the compact nature of uncultivated soil. The rates of denitrification have been observed to be higher in undisturbed soils than in ploughed soils (Rice and Scot Smith, 1982; Aulakh *et al.*, 1984 a, b; Linn and Doran, 1984; Staley *et al.*, 1990). Other possibilities for the higher denitrification rate are the high total carbon content and water-soluble organic carbon detected in the end of incubation time (Table 7.4) and the clay dispersion due to high ESP in this soil. As a result, soil hydraulic conductivity and infiltration rate will decrease and consequently lead to poor aeration.

Parkin and Tiedje (1984) observed that denitrification rates increased by 1 to 5 times when the O₂ concentrations in cores decreased from 20 to 5 %. They suggested that O₂ diffusion into aggregates and sites of denitrification might be a major factor controlling denitrification in soil. However, given the compact nature and low air porosity of Field No. 1 soil, a minor influence of ambient O₂ concentrations on denitrification in the cores would be expected. Soil type and tillage treatments were found to exert statistically significant influences on the concentration of N₂O measured in Scottish arable soils (Arah *et al.*, 1991).

If soil texture is the main factor limiting denitrification in these soils, we would expect to observe higher denitrification rates in clay or clay loam textured soil, i.e. in the topsoil cores from the field Nos. 1, 2, 3, 4 and No. 5 rather than in the sandy loam textured soil of the cores from field No. 6. This was expected since the particle size of the clay and clay loam soils is smaller, resulting in smaller pores, which are more likely to become anoxic zones with increasing moisture and/or

microbial CO₂ respiration. However, this was found not to be the case. In fact, the results show that higher denitrification rates were measured in sandy loam soil cores from field No. 6 than those measured in cores from the other fields (with the exception of that at field No.1). This might be attributed to the faster diffusion of N₂O out of these coarser soils and better acetylene diffusion into the cores. However, the differences were not statistically significant. The previous studies by Arah *et al.* (1991) observed that N₂O fluxes measured from a loam soil profile were higher than those from clay loam soil plots, thus supporting results obtained in this study. Arah *et al.* (1991) attributed the lower N₂O fluxes from the finer textured soil to further reduction of N₂O to N₂ within the soil.

The mean of the initial denitrification rates observed in soil cores from field No.1 was very significantly ($p < 0.001$) higher than those measured in soil cores from fields 2-6. This might be due to the high bulk density and low total porosity of this uncultivated fine textured soil, coupled with low soil permeability caused by destruction of the soil structure during clay dispersion. This may lead to restricted aeration and therefore the likelihood of more anoxic conditions and increased denitrification. These results are in agreement with other workers' observations (Arah *et al.*, 1991), which indicated that the lower N₂O production from the finer textured soil was attributed to the further reduction of N₂O to N₂ within the soil and its higher production from the coarse textured soil was due to the faster diffusion of N₂O out of these coarse soils.

Carbon dioxide production rates measured in cores from field No. 4 after the addition of carbon amendment were higher than those measured in the presence of natural carbon concentrations. This indicates that where carbon addition occurs, the formation of "hot spots" in the presence of a NO₃⁻ supply (section 7.2.4) results in denitrifier activity, and plays a more important role in decreasing O₂ concentrations. However, addition of carbon to soil cores from field No. 4 did not lead to significant increase in denitrification rates. This might be due to the low readily available organic carbon concentration (7.3 µg C g⁻¹ dry soil) which was added as a carbon amendment solution in the form of glucose.

Concentrations of water-soluble organic carbon in excess of 40 to 80 $\mu\text{g C g}^{-1}$ dry soil have been reported to be necessary before denitrification is observed in soils (Beauchamp *et al.*, 1980). In our study, it was found that water-soluble organic carbon concentrations observed in soil cores from the Axios Delta fields at the end of incubation time were too low with the exception of those in field No. 1 (Table 7.4), to meet the requirement of the observed denitrification rates. It was evident that available organic carbon might not be a controlling factor in topsoil denitrification at the Axios Delta fields.

N_2O concentrations in the cores decreased exponentially during phase 3, and gradually during phase 4. This could be attributed to the increased anoxic conditions promoting further reduction of N_2O to N_2 due to the failure of the C_2H_2 to completely inhibit N_2O reductase in the incubations. Another possibility is that N_2O concentrations declined due to leakage in the incubation systems.

Use of acetylene (C_2H_2) inhibition is unsuitable in long term studies due to its transient nature and adverse effects on soil microbial metabolism (Focht, 1982). Some reports have shown that C_2H_2 acts as a substrate for certain micro-organisms (Yeomans and Beauchamp, 1982; Topp and Germon, 1986). It is therefore possible that the high CO_2 concentrations observed in soil core incubations were formed by the oxidation of C_2H_2 , assuming that this phenomenon occurred and that the acetylene was consumed by micro-organisms. As a result, the remaining amount of acetylene is insufficient to inhibit N_2O reduction to N_2 . Thus, a failure of the C_2H_2 inhibition of N_2O reductase and consequently the further reduction of N_2O to N_2 is possibly the main reason that there was a period of sharp N_2O decrease.

At stage A, CO_2 concentrations of the soil core incubations of most fields increased sharply in the early period of incubation following acetylene addition. This is probably due to the O_2 depletion in the incubated soil cores and the observed N_2O concentration increase (exponential growth during phase 1). During stage B, CO_2 concentrations increased at a slower rate than during stage A and continued to increase slightly until the end of the incubation period. During this time period, N_2O concentrations started by increasing in a gradual manner after which a period of

sharp N_2O decrease was observed. N_2O concentrations then decreased at a slower rate and reached a near-stationary phase. It might be that at this stage (stage B) some of the denitrifier micro-organisms started to absorb some of the acetylene before the oxidation process took place and consequently left insufficient acetylene to inhibit N_2O reductase. All of these observations indicate that a decrease in oxygen diffusion into soil aggregates and into the sites of denitrification is a major factor controlling topsoil denitrification in the Axios Delta fields.

CO_2 concentration in the soil core incubations did not decrease during the entire incubation period. This indicates that no leakage occurred in the incubation systems. In addition, concentrations of CO_2 emission, from all topsoil incubations (figure 7.3 a-f) increased during the incubation period and were of a similar magnitude, except for soil cores from field No.1 which had a much higher CO_2 emission (Figure 7.3 a). This suggests that, apart from field No.1, a comparable amount of microbial activity was occurring in all incubations.

7.5 Overview

Overall, the results from this investigation indicate that the effect of soil texture on topsoil denitrification in the Axios Delta fields disagreed with the general view that higher denitrification rates are expected from fine-textured soil compared to those from coarser textured soil. Decrease in oxygen diffusion into soil aggregates and into sites of denitrification may be a factor controlling topsoil denitrification at the Axios Delta fields. The further reduction of N_2O into N_2 that occurred might be due to the failure of the C_2H_2 to completely inhibit N_2O reductase in soil core incubations. This was thought to be due to the microbial metabolism of C_2H_2 by denitrifiers. The maximum denitrification rate observed in the soil core incubations of the Axios Delta fields in the presence of natural carbon concentrations was $178 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ dry soil day}^{-1}$.

8 Conclusion

8.1 Wastewater irrigation

Taking into consideration the physical and chemical properties of the studied irrigated soil samples, the characteristics of the irrigation water qualities and method of irrigation used at the Galicos River Wastewater Treatment Project, as well as their interactions, this study indicates that four year furrow irrigation using wastewater did not have any adverse effects on the properties of the irrigated soil. Continuous use of this type of wastewater for irrigation purposes under the climatic conditions of the field site and the current management and land use practices in this project is therefore adequate. This may have important implications in areas that are arid and semi-arid where evaporation exceeds precipitation.

The quality of wastewater used for irrigation, in the course of four years in the Galicos River site, produced sodium saline soil. This kind of soil, subject to continuous irrigation with this wastewater, is not susceptible to becoming alkaline. Production of alkaline soils occurs through the hydrolysis of sodium clay and this type of reaction will only take place when either the irrigation water or the soil solution is sufficiently low in salts (Richards, 1954). This is not the case under the conditions detailed above.

Although there was a small decrease in the saturated hydraulic conductivity (K_{sat}) of soil irrigated with wastewater when compared to soil irrigated with well water, it was not a statistically significant difference. Furthermore, the decrease in K_{sat} was not sufficiently large enough to cause a restriction to soil water flow. These results were expected for two reasons. Firstly, the high salinity of wastewater would reduce the potential of Na^+ to deflocculate soil particles (Oster and Schroer, 1979). Secondly, the clay type of the soil studied is kaolinite (section 4.2.1). This type of clay is less sensitive to increases in the SAR of applied water as explained by McNeal and Coleman (1966) who concluded that the most labile soils were those

high in 2:1 layer silicates, especially montmorillonite, and the least labile were those high in kaolinite and sesquioxides.

Improvements in soil structure could be obtained by the addition of organic matter to the soil in order to increase aggregate stability and consequently reduce clay dispersion, since organic matter acts as a granulator of mineral particles (Brady, 1974).

8.2 Topsoil denitrification

Incubations with repacked and intact cores showed that topsoil denitrification plays an important role in reducing NO_3^- concentrations in the irrigated soils of the Axios Delta site, and in plots either irrigated with wastewater or well water in the Galicos River site. However, very low rates of denitrification of NO_3^- were observed to occur in repacked and intact soil cores from the Galicos River site in the presence of natural C concentrations. The average denitrification rate was $1.63 \mu\text{g N kg}^{-1}$ dry soil day^{-1} in repacked soil cores and $1.18 \mu\text{g N kg}^{-1}$ dry soil in intact soil cores from plots irrigated with wastewater. In soil cores from well water irrigated plots, denitrification rates were $1.26 \mu\text{g N kg}^{-1}$ dry soil day^{-1} in repacked soil cores and $1.05 \mu\text{g N kg}^{-1}$ dry soil day^{-1} in intact soil cores.

There are specific problems associated with quantifying total topsoil denitrification in laboratory incubations using the acetylene inhibition method. The major problem is that underestimation of the total denitrification is very likely to occur when using gas chromatographic (GC) analysis of nitrous oxide, together with the acetylene inhibition method. This happens because, firstly, N_2O has a tendency to undergo further reduction to N_2 if there is a deficiency of acetylene inhibition of N_2O reductase under severely anaerobic conditions. Secondly, microbial breakdown of C_2H_2 can occur, thus resulting in the alleviation of C_2H_2 inhibition of N_2O reductase. Thus, mass spectrometric techniques are a useful alternative for estimating total denitrification in irrigated topsoils, and measuring total ^{15}N -enriched denitrified gas in the absence of C_2H_2 . Another problem during GC analysis of N_2O in the presence of C_2H_2 comes from the overlapping of unlabelled N (native concentration of soil nitrate-N) and labelled N_2O -N fluxes. This can be overcome by taking

measurements of ^{15}N -enriched N_2O fluxes, using a continuous flow isotope-ratio mass spectrometer (CF-IRMS) for determining the specific N_2O -N denitrified from the applied ^{15}N -labelled fertilizer. CF-IRMS is a useful tool that can be used to identify the end product of denitrification in irrigated topsoils and to assess whether or not the irrigation with secondary wastewater contributes to the sources of N_2O emission to the atmosphere.

Topsoil denitrification of NO_3^- at the study sites can be viewed as an undesirable process. The end product of topsoil denitrification is likely to be N_2O rather than N_2 . This is because in topsoil N_2O readily diffuses into the atmosphere before further reduction to N_2 . This increases concerns over N_2O emissions into the atmosphere, since N_2O is a greenhouse gas and also causes depletion of the ozone layer (Groffman, 1995).

Irrigated soil quality in the field was found to be the main factor controlling denitrification in repacked and intact soil cores from the Galicos River Wastewater Treatment Project site. This may have important implications in areas such as land application of municipal wastewater where the addition of nitrogen and organic materials through irrigation water can lead to considerable pollution concerns. Addition of organic materials supplying available carbon to soil micro-organisms often results in enhanced denitrification (Feigin *et al.*, 1991). Denitrification in topsoil irrigated with wastewater may subsequently reduce NO_3^- concentrations in ground or surface waters to low levels.

Organic matter concentrations tend to be greatest at the soil surface, therefore a decrease in denitrification potential is observed down the soil profile (Rolston, 1981). High C concentrations lead to increased soil microbial activity and subsequently decreased O_2 concentrations, thus resulting in the formation of anoxic microsites that favour denitrification. However, the organic carbon content of wastewater and well water-irrigated soils is low, accounting for an average of 10 mg C g^{-1} dry soil. Therefore, the increase in denitrification rates in these irrigated soils may not reach levels likely to result in an environmental pollution hazard.

Crust formation induced on the surface layer of repacked and intact soil cores from the Galicos River site using a rain simulator had no significant effect on denitrification rate. This suggests that there are other factors more important than the presence of a crust layer in the promotion of the denitrification process. The application of surface tillage practices at the beginning of the irrigation season, such as very light ploughing of the topsoil, would remove any crust layer which might have been produced by the action of natural raindrop impact during the rainy season, and increase aeration. Ploughing and cultivation of the topsoil results in increased aeration and evaporation of moisture (Granli and Bøckman, 1994), and therefore reduces the likelihood of denitrification. Irrigation scheduling based on crop requirements can help to avoid over-irrigation and waterlogging, which create suitable conditions for denitrification.

The investigation of the effect of soil texture on total denitrification rates in intact soil cores from Axios Delta fields showed that the decrease in oxygen diffusion into soil aggregates and sites of denitrification may had a greater effect than soil texture.

8.3 Soil texture

A direct comparison of the effect of soil texture on denitrification losses between the clay soil cores of field No.1 and the clay, clay loam and sandy loam soil cores of the other fields was not possible. This was because of the clay dispersion in field No.1 due to a high percentage of exchangeable sodium (ESP) and the compact nature of its uncultivated soil. Clay dispersion leads to a reduced hydraulic conductivity of the soil profile, reduced infiltration rate of water, and also leads to poor aeration, which increases the denitrification potential.

Concentrations of N_2O emission measured in sandy loam soil cores from the Axios Delta fields were higher than those from soils having a clay or clay loam texture. Overall, the results are in agreement with other studies (Arah *et al.*, 1991), that indicated that the lower N_2O production from the finer textured soil was attributed to the further reduction of N_2O to N_2 within the soil, and its higher production from coarse textured soil was due to the faster diffusion of N_2O out of these coarse soils.

In sandy loam, clay or clay loam soil cores from the Axios delta fields, the concentrations of N_2O emission initially increased exponentially, followed by an exponential decrease. The initial increase in N_2O concentrations indicated that denitrification rates increased with the incubation time, probably due to a gradual decrease in oxygen diffusion into soil aggregates and denitrification sites. The exponential decrease in concentration of N_2O emissions observed in the middle-late stage of the incubation time could be the result of the microbial degradation of C_2H_2 with the concomitant alleviation of C_2H_2 inhibition of N_2O reductase.

Inhibition of N_2O reductase by 10 % by volume acetylene was found to be effective for nearly two weeks (13 days) in the incubation of sandy loam soil cores from the Axios Delta site. However, in other incubations of clay or clay loam soil cores it occurred for no more than one week. This indicates that acetylene addition in the concentration of 10 % by volume was not sufficient to inhibit reduction of N_2O in soil core incubations from the Axios Delta fields for the entire incubation time.

9 General Conclusion and Recommendations

9.1 General Conclusion

N losses through denitrification from irrigated soils at the Axios Delta fields and the Galicos River site were detected as indicated by N_2O emission measurements, but to a lesser extent than topsoil denitrification potential in irrigated soils as reported by other studies. This study has provided new and important data on topsoil denitrification potential at these experimental sites. Irrigated soil quality was probably the main factor controlling the topsoil denitrification in repacked and intact soil cores from the Galicos River site. Available organic carbon may also have been a factor regulating denitrification rates in repacked soil cores from the Galicos River site. However, crust formation had no significant effect on topsoil denitrification in this site. Decrease in oxygen diffusion into soil aggregates and into sites of denitrification may have been a factor affecting topsoil denitrification rates in intact soil cores from Axios Delta fields. Results from the investigation into the effect of soil texture on denitrification rates disagreed with the general view that higher denitrification rates are expected from fine-textured soil compared to those from coarser textured soil.

9.2 Recommendations

1. This study needs to be followed up over a long period of time with future work investigating topsoil denitrification down to the root zone depth. This will increase our understanding of microbial reduction of NO_3^- in irrigated soils at these experimental sites.
2. More research is needed to study topsoil denitrification at these field sites under different levels of organic carbon amendment concentrations in order to estimate the extent of topsoil denitrification rates at these field sites.
3. The continuous use of secondary municipal wastewater from Thessaloniki city for irrigation purposes is worthwhile.

4. Due to the biological and chemical pollution problems that might arise from wastewater irrigation, it is important that soils and crops produced are routinely examined.
5. In order to improve the productivity and quality of crops, it is advisable to invest in programmes for fertilization and crop protection.
6. Financial support and investment in highly experienced agricultural engineers, technicians and managers for this project development is important.

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11 APPENDICES

APPENDIX I Effect of total electrolyte concentration and sodium adsorption ratio of percolating solution on soil sodicity

A1.1 Introduction

One of the major factors affecting the suitability of water for irrigation is its sodicity hazard. Excessive sodium causes both crop toxicity and soil permeability problems. The electrical conductivity (EC) and sodium adsorption ratio (SAR) of irrigation water were found to be the most useful criteria for irrigation water classification (Richards, 1954; Rhoades *et al.*, 1992). Quirk and Schofield (1955) showed that the hydraulic conductivity (HC) of a given soil decreases with increasing exchangeable sodium percentage (ESP), provided that the electrolyte concentration is below a critical level (threshold level). Dispersion and swelling of clays within the soil matrix are interrelated phenomena, and either can reduce soil hydraulic conductivity. Swelling reduces soil pore sizes and dispersion clogs soil pores. Frenkel *et al.* (1978) concluded that the plugging of pores by dispersed clay particles is a major cause of reduced soil HC for surface soils irrigated with sodic waters. Swelling is not generally appreciable unless the ESP exceeds about 25 or 30 (Shainberg and Caiserman, 1971). However, dispersion can occur at ESP levels as low as 10 to 20 if the electrolyte level is $<10 \text{ mmol}_e \text{ l}^{-1}$ (Felhendler *et al.*, 1974). Rhoades (1968) reported that in irrigated soils, ESP and salinity are generally lowest at the soil surface and increase with depth through the root zone. The increased salinity with depth is usually sufficient to compensate for the increased level of ESP.

Iron and aluminium oxides, organic matter, clay content and dispersing agent (ESP) were found to be the main soil properties influencing aggregate stability (Goldberg *et al.*, 1988; Chappell *et al.*, 1999). Positive correlations ($r = 0.55$ and 0.52) were found between soil organic matter and percolation stability and between iron and aluminium oxides and percolation stability, respectively (Mbagwu and Auerswald, 1999).

Calcium carbonate also appears to have a direct cementing effect and contributes to structural stability (Rimmer and Greenland, 1976). Reduction in IR occurred at ESP values as low as 1 on a non-calcareous Netanya soil and 6.4 on a Nahal-Oz soil which contained 13 % CaCO_3 when the total electrolyte concentration (TEC) of the applied water was low (Agassi *et al.*, 1981).

The aim of this study was to investigate the effect of low and high SAR and EC levels of percolating solution (EC 0.1 or EC 2), (SAR 1 or SAR 20), on sodicity of a sandy silt loam soil collected from Greece.

A1.2 Materials and methods

Six intact sandy silt loam soil cores (7.3 cm in diameter and 5 cm in height) were collected from the Galicos River Wastewater Treatment Project in Greece at the beginning of the irrigation season (April 2000). These soil cores were irrigated by biologically treated wastewater over four years using a furrow. Additional soil cores were collected and analyzed for electrical conductivity, pH, CaCO_3 content and particle size distribution. The properties of the soil used are given in Table A1.1.

Table A1.1 Physical and chemical characteristics of the soil used. Figures represent averages (n=3).

Parameter	Mean
EC (dS m^{-1}) at 25 °C	0.71
pH	8.5
CaCO_3 %	4.5
Sand %	34.92
Silt %	48.19
Clay %	16.88

A1.2.1 Field site

The climate at the field site, and the specific soil sampling sites are described in Chapter 4 (section 4.2.1). The chemical characteristics of biologically treated wastewater are given in Table A1.2.

Table A1.2 Chemical properties of biologically treated wastewater used to irrigate soil in the field (Panoras *et al.*, 1999). Values represent averages (n=11).

Parameter	Biologically treated wastewater
TDS (mg l ⁻¹)	1645
pH	7.9
EC (dS m ⁻¹)	2.57
SAR (mmol _c l ⁻¹)	2.03
Na ⁺ (mg l ⁻¹)	377.4
K ⁺ (mg l ⁻¹)	23.4
Cl ⁻ (mg l ⁻¹)	609.4

A1.2.2 Leaching procedure

Three of the six soil cores were leached using different pore volumes from solutions of SAR 1 with salt concentrations of either 0.1 or 2 dS m⁻¹ until steady-state effluent compositions were achieved. The other three cores were leached with solutions of the same salt concentrations but with a SAR of 20. Pore volume from solutions was estimated using the volume of the leaching column occupied by soil and the percentage of pore space. Leachate was collected incrementally manually. The electrical conductivity and the concentrations of Na⁺ and Ca⁺⁺+Mg⁺⁺ soluble cations were measured periodically in the leachate and the SAR of the effluent was calculated. The electrical conductivity of the leachate samples was determined using standard techniques (U.S. Salinity Laboratory Staff, 1954). Na concentrations were determined using a flame photometer. Ca⁺⁺+Mg⁺⁺ concentrations were

measured by titration with ethylenediaminetetraacetate (Versenate) as described by Cheng and Bray (1951).

Soluble cation concentrations of soil water (leachate) were used to calculate SAR values using the following equation:

$$\text{SAR} = \frac{\text{Na}}{\sqrt{\frac{(\text{Ca} + \text{Mg})}{2}}} \quad (\text{Equation A1.1})$$

Where the ionic concentrations are expressed in $\text{mmol}_c \text{ l}^{-1}$

A1.3 Results and discussion

The change in leachate SAR of percolating solution from Greek surface soil cores were investigated and plotted as a function of total electrolyte concentrations (EC 0.1 or EC 2) and sodium adsorption ratio (SAR 1 or SAR 20) (Figure A1.1).

Soil sodicity depended on SAR and total electrolyte concentration of the percolating solution. The increase in total electrolyte concentration in the percolating solution (EC 2) of low SAR (SAR 1) reduced soil sodicity (clay dispersion) (Figure A1.1). However, this effect was not observed when soil was leached with a solution that had the same total electrolyte concentration (EC 2), and high SAR (SAR 20). When the soil was leached with a solution of low electrolyte concentration and low SAR (EC 0.1, SAR 1), the sodicity of the leachate increased. This might be because the $\text{Ca}^{++} + \text{Mg}^{++}$ concentrations in soil water had decreased to a low concentration (Table 3 a) and disaggregation may be enhanced even at low SAR levels. However, when soil was leached with the same solution of low electrolyte concentration (EC 0.1) but with high SAR (SAR 20), the sodicity of the leachate decreased (Figure A1.1). This might be attributed to the slight increase in $\text{Ca}^{++} + \text{Mg}^{++}$ concentration ($1.2 - 3.6 \text{ mmol}_c \text{ l}^{-1}$) in the leachate (Table A1.3c).

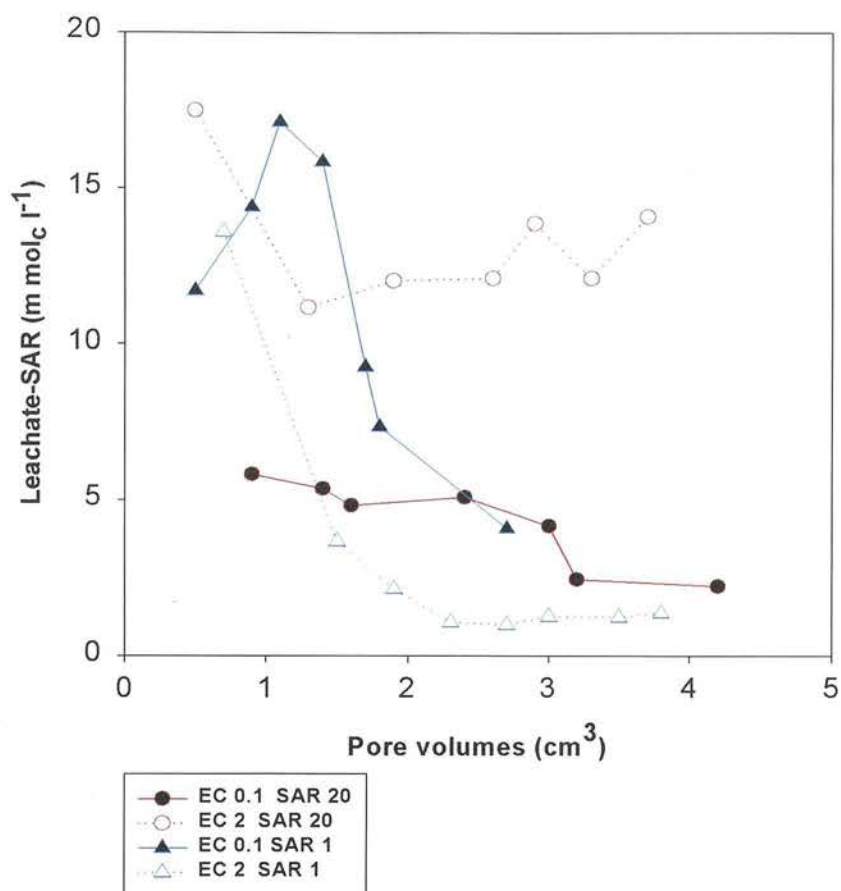


Figure A1.1 Sodidity of leachate from sandy silt loam soil with low and high sodium adsorption ratios (SAR) and electrical conductivity (EC) of percolating solution.

The increase in $\text{Ca}^{++} + \text{Mg}^{++}$ concentrations in the leachate indicates that CaCO_3 dissolution occurred during the leaching period. Calcium carbonate and high electrolyte concentrations have been found to be very effective against dispersion (Gupta *et al.*, 1984). Rhoades *et al.* (1968) showed that arid land soils release appreciable amounts of Ca^{++} and Mg^{++} to solution (typically increasing the concentration by 3-5 $\text{mmol}_e \text{ l}^{-1}$). A soil that readily releases salts during leaching with distilled water will be less susceptible to sodicity, will induce clay dispersion, and reduce hydraulic conductivity (Shainberg *et al.*, 1981). It has been found that lime (CaCO_3) dissolution affects the total electrolyte concentration of soil solution and clay dispersion (Rhoades *et al.*, 1968; Oster *et al.*, 1979; Rengasamy, 1983; Gupta *et al.*, 1984). Rengasamy (1983) observed that soil containing very small amounts of CaCO_3 (2 %) had very low dispersible clay, even when the ESP values were 15 and above.

The salt concentration (EC) and the concentrations of Ca^{++} and Mg^{++} in soil solution (leachate) increased as a function of increase in EC of percolating solution (Table A1.3b, d). The salt concentration (EC) in soil solution (leachate) was nearly of the same magnitude when the soil was leached with a solution of high electrolyte concentration (EC 2) with either low SAR or high SAR (SAR 1 or SAR 20) (Table A1.3b, d). However, the concentrations of Ca^{++} and Mg^{++} in soil solution (leachate) were greater when the soil was leached with the solution with a high electrolyte concentration and low SAR (EC 2, SAR 1) (Table A1.3b), compared with those leached with the same electrolyte concentration solution with high SAR (EC 2, SAR 20) (Table A1.3d). As a result, the increase in Ca^{++} and Mg^{++} concentration in soil solution under high electrolyte concentration and low SAR (EC 2, SAR 1), decreased soil sodicity.

Table A1.3 The concentration of soil water (leachate) under different concentrations of percolation solution.

Percolating solution		(a)			(b)			
		(SAR 1 EC 0.1)			(SAR 1 EC 2)			
Leachate	Pore volume	SAR mmol _c l ⁻¹	EC dS cm ⁻¹ at 25 °C	Ca+Mg mmol _c l ⁻¹	Pore volume	SAR mmol _c l ⁻¹	EC DS cm ⁻¹ at 25 °C	Ca+Mg mmol _c l ⁻¹
1	0.5	11.71	1.67	2.6	0.7	13.59	2.96	4.93
2	0.9	14.39	1.62	1.86	1.5	3.66	2.96	15.06
3	1.1	17.12	1.71	2.17	1.9	2.13	2.91	18.26
4	1.4	15.85	1.49	1.53	2.3	1.07	3.02	20.26
5	1.7	9.27	1.1	1.93	2.7	0.99	2.96	21.20
6	1.8	7.34	0.85	1.53	3.0	1.25	3.07	20.93
7	2.7	4.08	0.46	1.40	3.5	1.23	3.07	21.86
8					3.8	1.36	4.68	34.06

Table A1.3 continued.

Percolating solution		(c)			(d)			
		(SAR 20 EC 0.1)			(SAR 20 EC 2)			
Leachate	Pore volume	SAR mmol _c l ⁻¹	EC dS cm ⁻¹ at 25 °C	Ca+Mg mmol _c l ⁻¹	Pore volume	SAR mmol _c l ⁻¹	EC dS cm ⁻¹ at 25 °C	Ca+Mg mmol _c l ⁻¹
1	0.9	5.81	1.25	3.2	0.5	17.49	3.59	5.40
2	1.4	5.34	0.76	2.13	1.3	11.16	3.02	5.86
3	1.6	4.81	0.82	1.20	1.9	12.02	3.64	7.80
4	2.4	5.07	0.42	1.26	2.6	12.10	3.22	5.73
5	3.0	4.15	0.59	1.93	2.9	13.86	4.27	8.0
6	3.2	2.44	0.69	3.66	3.3	12.10	4.06	8.06
7	4.2	2.23	0.67	3.4	3.7	14.08	3.33	5.40

A1.4 Conclusion

Results presented here indicate that increases in salt concentrations of percolating solution, and $\text{Ca}^{++} + \text{Mg}^{++}$ concentrations in soil solution, decreased the soil sodicity which consequently reduced clay dispersion in the type of soil under study.

APPENDIX 2 Nitrous oxide production from Libyan, English and Greek soil aggregates amended by organic carbon

A2.1 Introduction

In natural soils, individual particles, such as clay and silt, adhere together to form complex aggregates of varying sizes and shapes. In a structured soil (soil containing aggregates) there are large pores between aggregates and small pores within aggregates. Aggregates may remain virtually waterlogged for extended periods after the pores between them have drained (Thomasson and Robson, 1967). The amounts of various soil constituents such as organic matter, iron and aluminium oxides, percent and mineralogy of clay fraction and calcium carbonate are the main factors controlling aggregate stability (Giovannini and Sequi, 1976; Frenkel *et al.*, 1978; Goldberg *et al.*, 1988; Shainberg, 1992).

It has been shown by Currie (1961) that gaseous diffusion through aggregated, fine-textured soils occurs much more readily through the relatively large inter-aggregate pores than through the pores within the aggregates. The size of the soil aggregates has a major effect on denitrification and N_2O production. Large aggregates will become anaerobic more easily than small ones under identical conditions, but penetration of NO_3^- and degradable organic materials into large aggregates can be slow (Granli and Bøckman, 1994). Apart from O_2 , the other factor which limits denitrification is often organic carbon. In many arid zone soils, where organic matter levels are low, the rate of denitrification may often be C limited. Arah and Smith (1989) included aggregate size in their mathematical model for denitrification. Model calculations indicated that the denitrification rate increased strongly due to O_2 limitation as the aggregate size increased from 5 mm to 30 mm.

The objective of this study was to assess the effect of different aggregate sizes using Libyan, English and Greek soil aggregates, on N_2O production in the presence of carbon amendment.

A2.2 Material and methods

Twelve surface soil samples (0-30 cm depth) were collected from the Agricultural Research Centre's El-Marj experimental station, Libya located at 32° 3' N latitude, 20° 4' E longitude in the east of Libya in March 1999. In addition, one soil sample was obtained from Greece and three soil samples were obtained from England.

A2.2.1 Field site

The Libyan clay type is illite. These soils have organic matter contents in the range 0.29 – 1.79 %. The climate of the El-Marj experimental station is Mediterranean, and semi-arid, with hot dry summers and warm rainy winters, and the mean annual precipitation is about 400 mm. Crops are rainfed, not irrigated. The English soil type is montmorillonitic clay, and the Greek soil type is kaolinitic clay.

A2.2.2 Soil aggregate preparation and analysis

All the soil samples were gently sieved into two aggregate size classes (<4.75 and >4.75 mm diameter). These were used to assess the influence of the size of the soil aggregate on N₂O production following amendment with carbon. The fine particles of air dried soil samples were then passed through a 2-mm sieve and analysed for electrical conductivity, pH, soluble and exchangeable cations, chloride concentration and cation exchange capacity, as described in Chapter 4 (Table 4.2). The properties of the soils are given in Table A2.1.

The layout of the experiment is given in Table A2.2. Air dried aggregates from both aggregate sizes (<4.75 and >4.75 mm diameter) were weighed and placed in a plastic lid over Whatman No. 42 filter paper (Figure A2.1). This plastic lid was placed in the top of an empty steel ring (4 cm high) inside a half-litre incubation vessel containing 80 ml (\cong 1 cm depth) of glucose and nitrogen fertilizer solution (1 g l⁻¹ glucose and 50 mg N l⁻¹ as Ca(NO₃)₂). The top end of a piece of tissue paper was fixed under the filter paper and the bottom end was immersed in the nitrogen and glucose solution. The tissue paper was used to amend soil aggregates with N fertilizer and carbon solution by capillary rise (Figure A2.1). Each treatment was

replicated twelve times for illitic Libyan clay soil (one from each sample), three times for the montmorillonitic English clay soil (one from each sample), and three subsamples were taken from the Greek soil sample (Table A2. 2), with one replicate taken from each subsample.

Incubation vessels were closed and placed in an incubator at a temperature of 15 °C without acetylene addition throughout the experimental period (21 days), except during sampling (a few minutes for each sampling). There was no flushing over the entire experimental period. A gas chromatograph (GC) fitted with an electron capture detector (ECD) and thermal conductivity detector (TCD) was used to determine the concentrations of N₂O and CO₂ respectively. Gas from the head space of the incubation vessels was sampled after 1, 2, 3, 7, 14 and 21 days from the start of the incubation. At each sampling, 1 ml of gas from the head space of the incubation vessels was taken out using a syringe and injected into gas chromatograph manually. At the end of the incubation time, the incubation vessels containing the Greek soil aggregates (large and small in size) were flushed and followed by the addition of 10 % by volume acetylene, and incubated for two more days in the same incubator. After this, the head space was sampled for N₂O and CO₂ analysis. To investigate the effect of the addition of readily organic carbon on N₂O emission, the same experiment was repeated without the addition of carbon amendment. Soil aggregates were amended with 80 ml of nitrogen fertilizer solution (50 mg N l⁻¹ as Ca(NO₃)₂) only. Incubation vessels were incubated for the same time period (21 days) without acetylene addition and without flushing. Gas samples for N₂O and CO₂ were taken after 1, 2, 3, 7, 14 and 21 days from the start of the incubation. As in the first experiment, the vessels with the Greek soil aggregates were flushed at the end of the incubation time (21 days) and 10 % by volume of acetylene was added and the incubation was continued for two more days as before.

Table A2.1 Chemical analysis of soil samples. Figures represent averages (n=3).

Parameter	Illitic soil (Libyan soil)	Kaolinitic soil (Greek soil)	Montmorillonitic soil (British soil)
pH	7.54	8.6	5.74
EC (dS m ⁻¹)	0.60	2.02	1.09
Soluble cations (mmol _c l ⁻¹)			
Na ⁺	1.45	20.21	0.71
K ⁺	1.00	0.18	0.42
Ca ⁺⁺	2.92	1.96	7.51
Mg ⁺⁺	1.36	3.16	0.70
Soluble anions (mmol _c l ⁻¹)			
Cl ⁻	1.27	1.93	1.10
Exchangeable cations (mmol _c 100 g ⁻¹ soil)			
Na ⁺	0.20	1.85	0.08
K ⁺	1.16	0.3	0.45
Ca ⁺⁺	17.29	3.0	16.66
Mg ⁺⁺	2.74	5.4	1.07
CEC (mmol _c 100g ⁻¹ soil)	22.11	11.20	23.76
ESP (%)	0.90	16.51	0.34

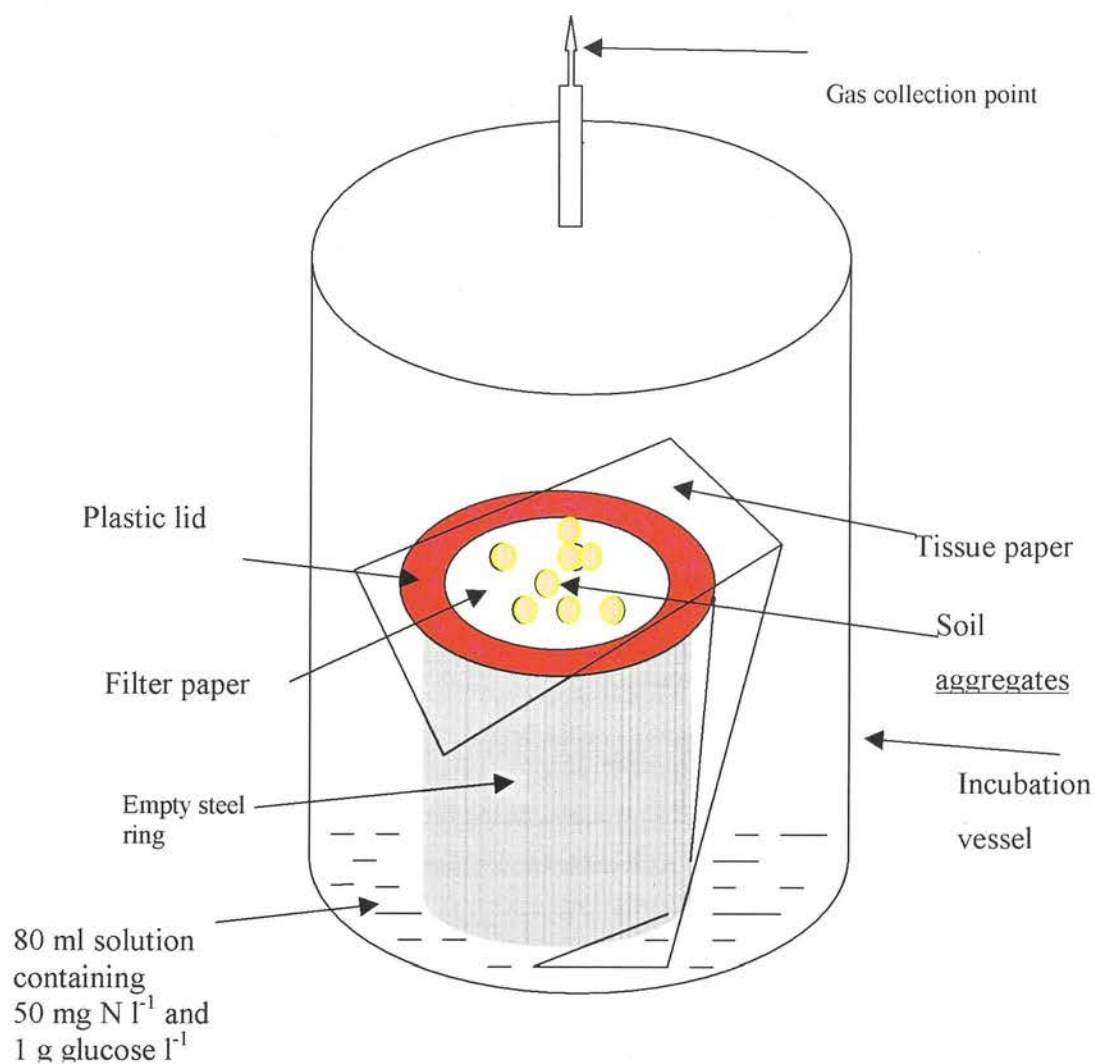


Figure A2.1 A diagram of the closed incubation vessel used to investigate denitrification in soil aggregates.

Table A2.2 Layout of experiment

Soil source	Number of replicates	Aggregate size	Treatment
Libya	12	Large (>4.75 mm)	+N, C- (Large C-)
	12	Small (<4.75 mm)	+N, C- (Small C-)
	12	Large (>4.75 mm)	+N, C+ (Large C+)
	12	Small (<4.75 mm)	+N, C+ (Small C+)
England	3	Large (>4.75 mm)	+N, C- (Large C-)
	3	Small (<4.75 mm)	+N, C- (Small C-)
	3	Large (>4.75 mm)	+N, C+ (Large C+)
	3	Small (<4.75 mm)	+N, C+ (Small C+)
Greece	3	Large (>4.75 mm)	+N, C- (Large C-)
	3	Small (<4.75 mm)	+N, C- (Small C-)
	3	Large (>4.75 mm)	+N, C+ (Large C+)
	3	Small (<4.75 mm)	+N, C+ (Small C+)

where

Large C- = Large soil aggregates (>4.75mm) not amended with carbon.

Small C- = Small soil aggregates (< 4.75mm) not amended with carbon.

Large C+ = Large soil aggregates (>4.75mm) amended with carbon.

Small C+ = Small soil aggregates (< 4.75mm) amended with carbon

A2.3 Results

N₂O production and CO₂ evolution rates from both size classes of Libyan, English and Greek soil aggregates are shown in figures A2.2 and A2.3.

N₂O production rates varied between the three soils under study. The highest N₂O fluxes under all treatments were observed from Greek soil aggregates (Figure A2.2 c) while the N₂O rates from Libyan soil aggregate incubations were the lowest (Figure A2.2 a). N₂O production rates from Libyan and Greek soils were observed to be higher when the size of aggregates were small, and decreased as aggregate size increased (Figure A2.2 a, c). However, N₂O production rates from English soils were much higher when the size of soil aggregates were large compared with soil aggregates that were small in size (Figure A2.2 b). The highest N₂O rates were

observed from carbon amended soil aggregate incubations (Figure A2.2 a, b, c). N_2O fluxes from Greek soil aggregates increased after acetylene addition under all treatments on day 23 (Figure A2.2 c). The highest N_2O production rates observed in the incubations of Libyan, English and Greek soil aggregates were 169, 466 and 1140 $\mu\text{g N kg}^{-1}$ dry soil day^{-1} respectively. The highest CO_2 evolution rates were not associated with the highest N_2O production rates (Figures A2.2, A2.3).

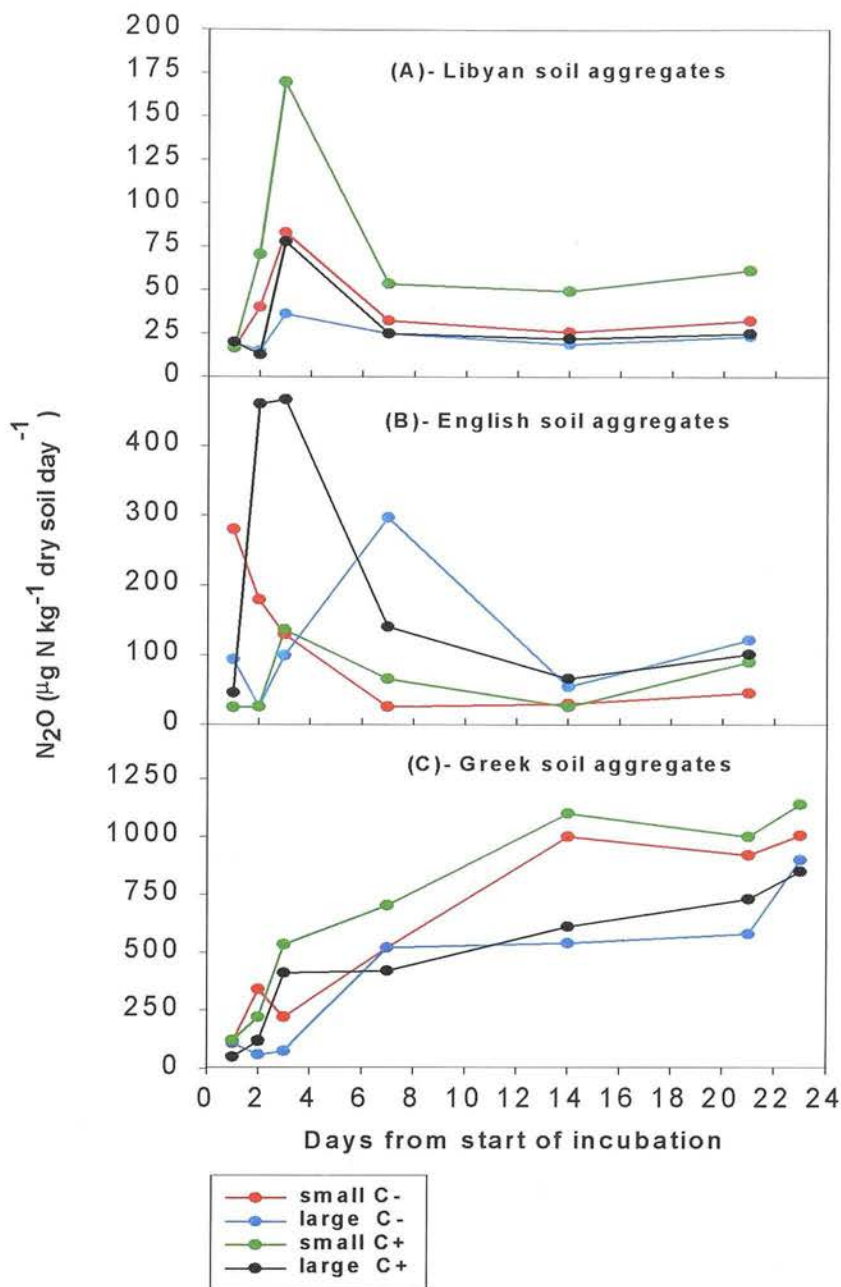


Figure A2.2 N_2O production rates from two soil aggregate sizes ; large (>4.75 mm diameter) and small (< 4.75 mm diameter), from; (A) Illitic Libyan soils; (B) montmorillonitic English soils; and (C) kaolinitic Greek soils, in the presence and absence of carbon amendment (C^+ , C^-). Each point represents the mean value of twelve replicates for illitic Libyan clay soil, the mean value of three replicates for montmorillonitic English clay soil and the mean of three replicate subsamples of the kaolinitic Greek clay soil.

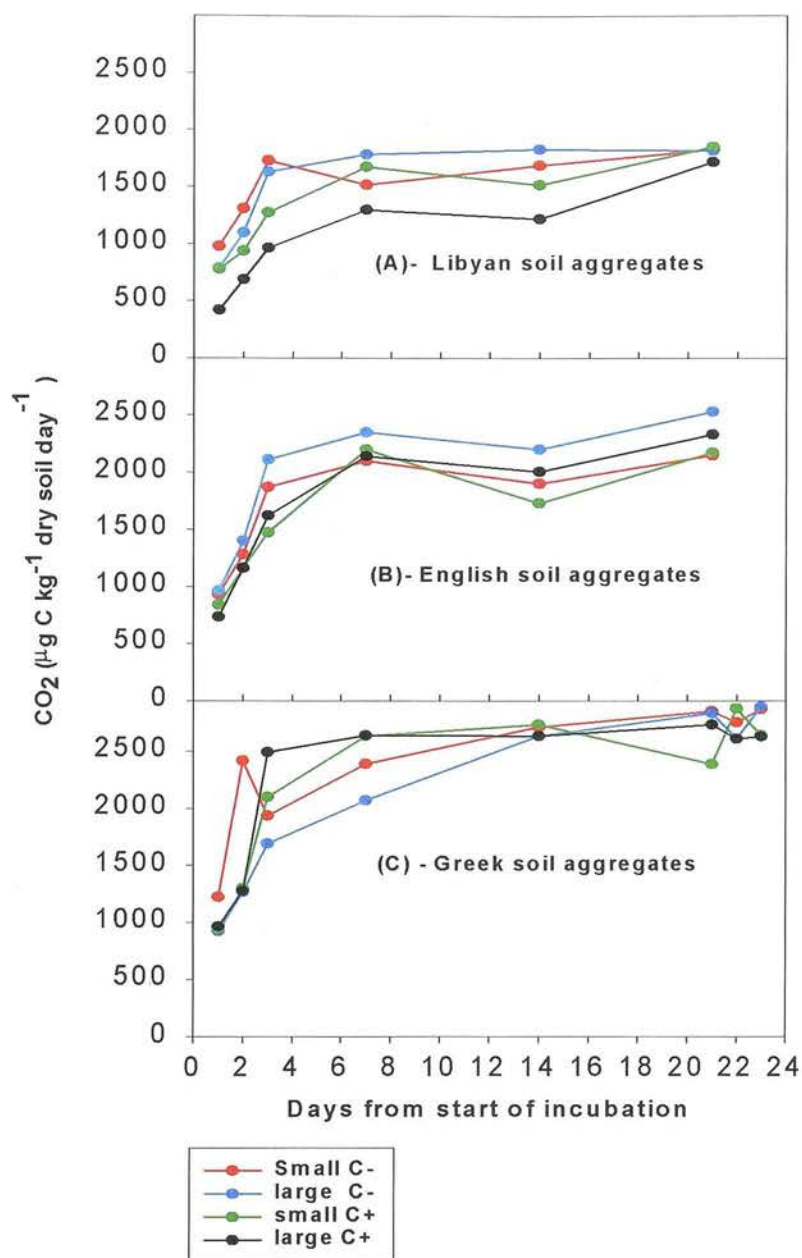


Figure A2.3 CO₂ evolution rates from two sizes of soil aggregates (large and small) (>4.75 & < 4.75 mm) from; (A) Illitic Libyan soils; (B) montmorillonitic English soils; and (C) kaolinitic Greek soils in the presence and absence of carbon amendment (C+, C-). Each point represents the mean value of twelve replicates for illitic Libyan clay soil, the mean value of three replicates for montmorillonitic English clay soil, and the mean of three replicate subsamples of the kaolinitic Greek clay soil.

A2.4 Discussion

For the Libyan and Greek soils, the higher N_2O production rates were observed in the small aggregates. This is probably due to the fast penetration of NO_3^- and degradable organic materials into small aggregates (Granli and Bøckman, 1994). The previous studies by Seech and Beauchamp (1988) observed that the denitrification rate was 2 to 3 times higher in small aggregates (<0.25 mm) than in large aggregates (5 - 20 mm). They attributed this to the low carbon substrate availability in the larger aggregates. In contrast, N_2O production from the large English soil aggregates was higher than that from small aggregates. This might be attributed to the more widespread occurrence of anaerobic centres in the larger aggregates. This was also observed by Nömmik (1956) who found higher N_2O production rates in soil aggregates <0.3 mm and >4 mm than in aggregates of intermediate size.

Lower N_2O production rates were observed from Libyan soil aggregate incubations under all treatments. This might be due to increased anoxic conditions promoting further reduction of N_2O to N_2 in the absence of acetylene (C_2H_2). One possible explanation for the high reduction of N_2O to N_2 in Libyan soil aggregate incubations is that O_2 diffusion into soil aggregates is slower in the presence of the illitic clay type of the Libyan soil compared with the kaolinitic and montmorillonitic type of clay of the Greek and English soils, respectively.

The increase in N_2O fluxes observed at day 23 from the incubations of Greek soil aggregates after acetylene addition under all treatment conditions was evidence for the reduction of N_2O in the absence of acetylene addition. Results observed from this study indicate that the size of the soil aggregate and readily available organic carbon had an effect on N_2O production rates, and suggest the importance of clay type mineralogy and acetylene inhibition on N_2O reduction under more anaerobic conditions.

APPENDIX 3 The effect of exchangeable sodium percentage (ESP) on N₂O emission from illitic Libyan soil aggregates

A3.1 Introduction

The presence of high sodium content in irrigation water leads to an increase in the exchangeable sodium percentage (ESP) of irrigated soil. Soil with a high exchangeable sodium percentage (ESP) will be subject to soil structure breakdown leading to swelling of clay domains and clay dispersion, and as a result will cause the hydraulic conductivity of the soil profile and infiltration rate (IR) to decrease.

Due to slaking of surface soil aggregates, soil swelling leads to lower air filled porosity (poor aeration), and enhanced nitrogen losses through denitrification. This is because denitrification only occurs under conditions where the demand for O₂ of the soil microbial respiration is not met by the diffusion of O₂ into soil aggregates and through the soil profile.

The sodicity of irrigation water is the main factor affecting crop toxicity and soil permeability (Richards, 1954). The potential hazard of irrigation water with a high sodium adsorption ratio on soil physical properties has been widely investigated (Richards, 1954; Crescimanno *et al.*, 1995). Richards (1954) suggested that a SAR value of 10 and an ESP value of 15 % are the critical levels above which soil structure is destroyed. As a result of the loss of structure, soil permeability problems occur. However, Crescimanno *et al.* (1995) reported that significant soil degradation can occur even in a 2 - 5 ESP range at a low cationic concentration. In poor soil structure, a higher ESP range enhances aggregate breakdown (Shainberg *et al.*, 1992).

The objective of this experiment was to measure the extent of N₂O production from illitic soil aggregates (Libyan soils) at a range of ESPs (high and low) in the presence of carbon amendment.

A3.2 Materials and methods

Six surface soil samples (0-30 cm depth) collected from the Agricultural Research Centre El-Marj experimental station, Libya (Appendix 2) were used in this study.

A3.2.1 Field site

The field site location, climate and soil properties are described in Appendix 2 (section A2.2.1, Table A2. 1).

A3.2.2 Soil aggregates preparation and analysis

Air dried Libyan soil aggregates (>4.75 mm diameter) were taken from each soil sample and weighed. Soil aggregates from each sample were packed with coarse sand in a soil leaching column. One set of three columns were leached with 100 ml of 0.1 M NaCl solution. This procedure was repeated daily for five days followed by leaching with the same amount of distilled water daily for three days. This was to produce soil aggregates with a high exchangeable sodium percentage (ESP) which would swell when leached with a good quality of water resulting in few air-filled pores, and as a result, give high N₂O and denitrification losses. The other three columns were leached with 100 ml of 0.01 M CaCl₂ in a similar way, followed by leaching with the same volume of distilled water, as before. This was to produce soil aggregates with a low exchangeable sodium percentage (ESP) which would not swell when leached with a good quality of water, leaving more aerated soil aggregates and leading to less N₂O and denitrification losses. The leachate of each leaching sequence was collected manually and measured, and the total soluble salt concentration determined periodically using a TDS-meter. The electrical conductivity was calculated as follows:

$$EC \text{ (dS m}^{-1}\text{)} = (\text{mg kg}^{-1} * f_t) / 640 \quad \text{(Equation A3.1)}$$

(Richards, 1954)

where

f_t = Temperature correction factor

In each leaching sequence using distilled water, the suspended clay contents in the various leachate increments were determined by transferring 10 ml of leachate into a 25 ml empty beaker of a known weight (W1), then placing it in an oven overnight at 105 °C. The beaker was removed from the oven, cooled in a desiccator containing an active desiccant, and weighed again (W2). The difference in weight gave the amount of suspended clay in each leaching sequence. The corresponding percentage of clay, of the initial weight of soil aggregates was calculated.

Following the leaching process, the leaching columns were placed in an oven for five days at 60 °C. The weights of the soil columns were taken every day. At the end of day five, soil aggregates and coarse sand were removed from the leaching columns and left to air dry at room temperature for four days. Air dried soil aggregates were then collected, weighed and incubated with 80 ml of glucose and nitrogen fertilizer solution. Na-saturated soil aggregates were incubated with 1 g l⁻¹ glucose and 50 mg N l⁻¹ as NaNO₃, while Ca-saturated soil aggregates were incubated with 1 g l⁻¹ glucose and 50 mg N l⁻¹ as Ca(NO₃)₂. The incubation vessel was described in Appendix 2 (Figure A2.1). Incubation vessels were closed and placed in an incubator at a temperature of 15 °C without acetylene addition for 7 days, except during sampling (a few minutes for each sampling). There was no flushing over this time period. A gas chromatograph (GC) fitted with an electron capture detector (ECD) and a thermal conductivity detector (TCD) was used to determine the concentrations of N₂O and CO₂ respectively. Gas from the head space of the incubation vessels was sampled after 1, 2, 3, 7, and 14 days from the start of incubation. For each sampling, 1 ml volumes from the headspace of the incubation vessels were taken periodically using a syringe and injected manually. Following seven days' incubation, vessels were flushed with 10 % by volume of acetylene, and the incubation continued for one more day, after which the gas was sampled for N₂O and CO₂.

A3.3 Results and discussion

The salt balance for CaCl₂ and NaCl in the soil leaching columns during leaching is shown in Figures A3.1 and A3.2 respectively. These figures indicate that soil

aggregates were adjusted to the high and low exchangeable sodium percentage (ESP) levels.

The suspended clay started to appear in the effluent with distilled water leaching. This is good evidence for the susceptibility of clay to dispersion at dilute concentrations of percolating solution. The percentage of suspended clay in the leachate of Na-saturated soil aggregates leached with distilled water increases with increasing salt concentrations of the leachate solution.

The total amounts of clay removed from the columns of the Na-saturated soil aggregates leached with distilled water were 3.54, 4.93 and 6.25 g, which corresponds to 15.06, 21.55 and 30.42 % of the initial soil aggregate in the column, while the amounts of clay removed from the columns of the Ca-saturated soil aggregates leached with distilled water were not more than 2.7 % of the initial aggregate weight.

The electrical conductivity (EC) of the leachate (from leaching with distilled water) decreased during the leaching sequence. This indicates that this illitic clay soil (Libyan soil) is not releasing salt during leaching with distilled water and may be highly susceptible to clay dispersion as a response to low exchangeable Na. The amounts of clay removed from the columns of the Na-saturated soil aggregates leached with distilled water support this hypothesis. Soils that readily release salt during leaching with distilled water will be less susceptible to sodicity (Shainberg *et al.*, 1981).

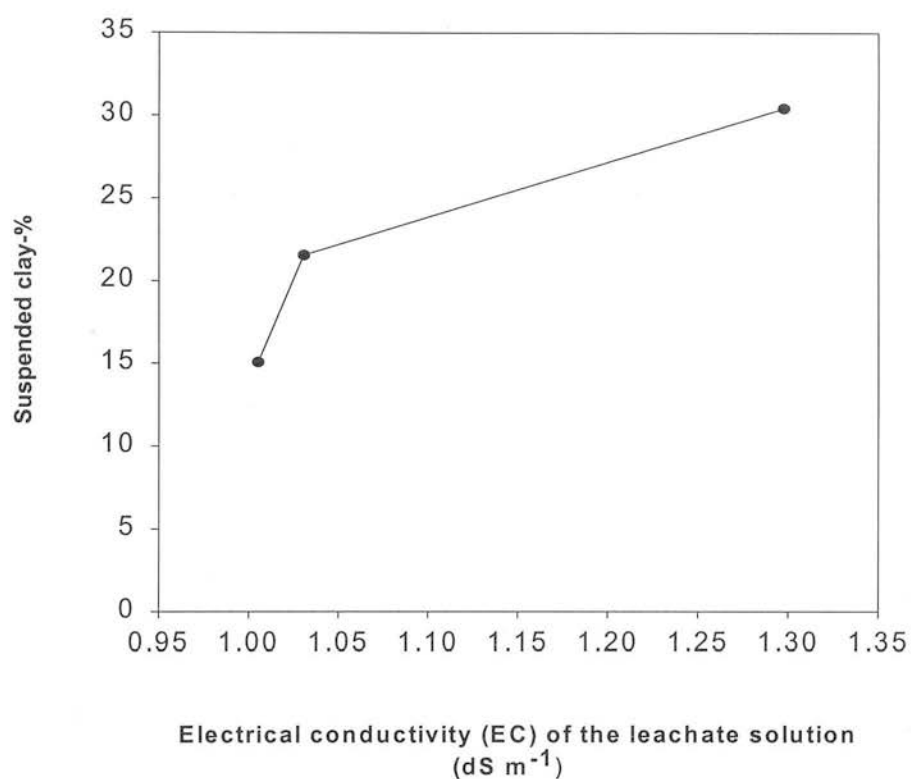


Figure A3.1 The percentage of suspended clay in the leachate of Na⁺-saturated soil aggregates versus salt concentrations of the leachate solution. Each point represents the mean value of three leaching sequences with distilled water, for one replicate.

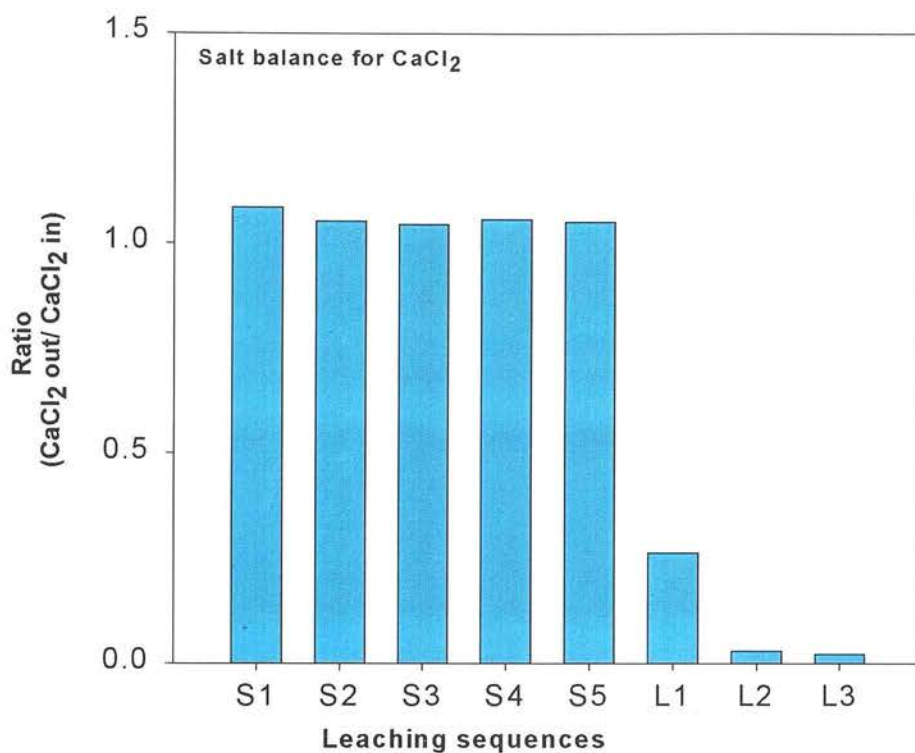


Figure A3.2 Salt balance in leaching soil columns of illitic soil aggregates (>4.75 mm diameter) (Libyan soil) leached daily with 100 ml of (0.01M CaCl₂) solution for five days (S1, S2, S3, S4, S5) followed by leaching daily with the same amount of distilled water for three days (L1, L2, L3). The CaCl₂ concentration at each leaching sequence represents the mean value of three replicates.

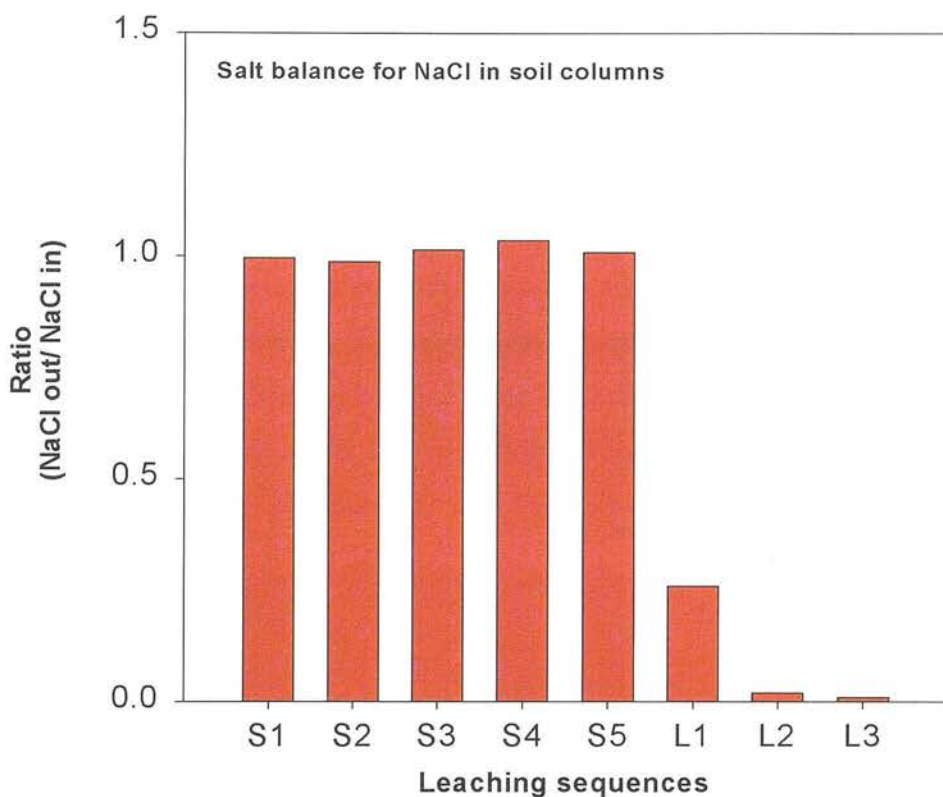


Figure A3.3 Salt balance in leaching soil columns of illitic soil aggregates (>4.75 mm diameter) (Libyan soil) leached daily with 100 ml of (0.1M NaCl) solution for five days (S1, S2, S3, S4, S5) followed by leaching daily with the same amount of distilled water for three days (L1, L2, L3). NaCl concentration at each leaching sequence represents the mean value of three replicates.

N₂O emission rates and CO₂ production in incubations of treated soil aggregates in the presence of added carbon amendment are shown in Figure A3.3.

N₂O production rates measured in Ca-saturated soil aggregate incubations were nearly the same magnitude as the N₂O rates measured in the incubations of the same aggregate size, but not saturated either with sodium or calcium (control) (see Appendix 2). The highest N₂O production rates were observed from Na-saturated soil aggregates (Figure A3.3) compared with those measured in Ca-saturated soil aggregate incubations. This might be because of dispersed clay, and swelling of Na-saturated clay soil aggregates after leaching with distilled water. This leads to poor aeration and an increase in nitrogen losses through denitrification. Acetylene addition seven days after incubation caused N₂O production rates start to increase. This is a good indication for the inhibition of N₂O reduction. The highest CO₂ production rates were not associated with the N₂O emission rates in soil aggregate incubations.

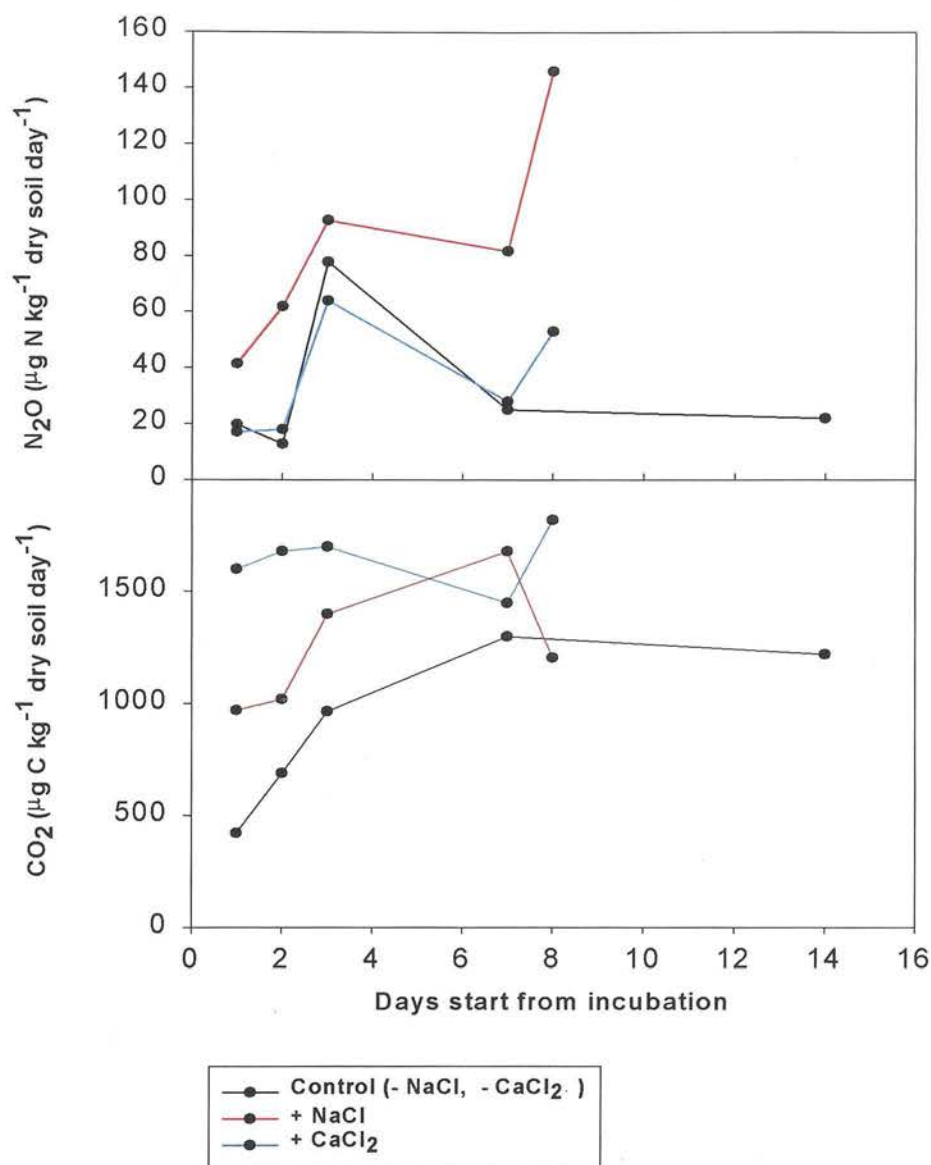


Figure A3.4 N_2O emission and CO_2 -production rates during the incubations of either Na or Ca-saturated illitic soil aggregates (>4.75 mm diameter) (Libyan soils) and non salt saturated aggregates (control) in the presence of carbon amendment. Each point represents the mean value of twelve replicates for control treatment and three replicate under + NaCl and + CaCl₂ treatments.

A3.4 Conclusion

These results indicate that the clay dispersibility of the illitic soil (Libyan soils) was very sensitive to the level of exchangeable sodium and to the salt concentration of the percolating solution, and suggests that increasingly higher concentrations of salt were needed in the percolating solution to prevent clay dispersion. The higher ESP enhanced the swelling and clay dispersion of aggregates. As a result, more anaerobic conditions occurred and led to the further reduction of N_2O to N_2 .

Appendix 4 The effect of soil water potential on N₂O production rate

A4.1 Introduction

Soil moisture is a critical factor in the regulation of biological denitrification, because O₂ diffuses 10⁴ times more slowly in water than in air. Soil microbial activity is known to be strongly influenced by soil water content (Parr *et al.*, 1981). Bacteria are only capable of moving rapidly over surfaces when the thickness of the water film is comparable to that of the bacterial cell (Lynch, 1988). Several forces act upon soil water and contribute to its total potential, including gravitational potential, solute potential, and matric potential. Irregular pore geometry and discontinuity, and variations in texture and mineralogy are the primary soil properties influencing soil water retention. Therefore, irrigation with water of different qualities and different irrigation systems may create zones with high water content and a high O₂ demand where denitrification can occur, even at a relatively low water potential of the bulk soil (Comfort *et al.*, 1988). Different methods are used for measuring soil water potential such as porous plate methods (either as a pressure plate or as a suction plate), and pressure membranes. All of these methods suffer from slow equilibration times, a limited range of measurements, or both. The filter-paper method has also been used for measuring soil suction. Gardner (1937) first used the filter-paper technique. This method has limited equipment requirements, and a wide range of potentials can be measured (from -1 kPa to -100 MPa) (Fawcett and Collis-George, 1967).

The aim of this study was to investigate the effect of soil water potential on N₂O production rate in intact soil cores from the Galicos River Wastewater Treatment Project and from the Axios Delta fields, in northern Greece.

A4.2 Material and Methods

Twelve intact soil cores (7.3 cm in diameter, 5 cm in height) containing 271.9 g dry soil, were collected from the Galicos River Wastewater Treatment Project at the beginning of the irrigation season (April 2000). Four soil cores were taken from each of the selected plots which had been furrow irrigated either with wastewater (Q1), biologically treated wastewater (Q2) or with well water (Q3) over four years. Another twelve intact soil cores (7.3 cm in diameter, 5 cm in height) were collected at the same time from six fields at the Axios Delta in Greece which had been irrigated by flooding. Two soil cores were collected from each field. Some of the chemical properties of the different irrigation water qualities are given in Chapter 4 and in Appendix 1.

A4.2.1 Study sites and climate

There were as described in Chapters 4 and 7.

A4.2.2 Soil analysis

In this experiment, a filter-paper technique was used to measure the soil water matric potential of intact cores.

A4.2.2 Equilibration process

Whatman no. 42 filter paper was cut into pieces that were 7.3 mm in diameter. One piece was placed in direct contact with the top end of an intact soil core and another piece was put at the bottom end. A polyethylene sheet was put on top of each piece of filter paper to ensure good contact. This is similar to a porous plate. The soil core and filter paper were sealed at the top and bottom ends using plastic lids to prevent evaporation, and then allowed to equilibrate for six days at a room temperature of 25 °C. Once the filter papers had reached equilibrium, the papers were then removed with tweezers. Any soil adhering to the papers was lightly brushed off and the papers weighed immediately. The paper wet and dry mass (after drying overnight at 105 °C) were used to determine the gravimetric moisture content of the filter papers (g g^{-1}). The average water content of the filter paper was then

calculated, and the soil matric potential calculated from the following equations reported by Deka *et al.* (1995):

$$\text{Log}_{10} (-\psi_m) = 5.144 - 6.699M, \psi_m < -51.6 \text{ kPa (wet soil)}$$

$$\text{Log}_{10} (-\psi_m) = 2.383 - 1.309M, \psi_m > -51.6 \text{ kPa (dry soil)}$$

where

M = water content of filter paper (g g^{-1}).

After that, the water release curves for soils treated with each irrigation water quality were plotted as the relationship between matric soil water potentials measured using the filter-paper technique and their moisture content, (Figure A4.1). Each soil core was then irrigated with 20 ml of glucose and nitrogen fertilizer solution (1 g l^{-1} glucose and 50 mg N l^{-1} as calcium nitrate). This solution gave a concentration of $3.6 \mu\text{g N g}^{-1}$ dry soil and $29.4 \mu\text{g C g}^{-1}$ dry soil. Infiltration rate was estimated by counting the time taken for this volume of solution to percolate.

A4.2.2.2 Closed incubation system and incubation of the intact soil cores

Following irrigation, soil cores from the Galicos River Wastewater Treatment Project were incubated in incubation vessels as described in Appendix 2 (Figure A2 1). Incubation vessels were closed and placed in an incubator at a temperature of 15°C without acetylene addition for 58 hours except during sampling (a few minutes for each sampling). The gas was sampled after 12, 34 and 58 hours from the start of incubation. There was no flushing over this time period. 1 ml volumes from the head space of the incubation vessels were taken and analyzed for N_2O and CO_2 after 12, 34 and 58 hours from the start of incubation. The first sample was taken 12 hours after the start of the incubation period.

Following incubation and gas measurements, soil cores were placed together on the tension table at controlled room temperature ($22 \pm 2^\circ\text{C}$) and drained to -200 kPa matric potential. This was to investigate the effect of soil water potential on N_2O production. After that, soil cores were irrigated with another 20 ml from the same

glucose and nitrogen solution and incubated without acetylene for three days. 1 ml from the head space of the incubation vessels was taken and analyzed for N_2O and CO_2 after 12, 34 and 58 hours from the start of incubation. Incubation vessels were flushed for 20 minutes after each sampling period.

Soil cores from the Axios Delta fields were also irrigated with 20 ml of glucose and nitrogen solution. The infiltration rate (IR) was estimated and the soil cores were incubated in the same incubator for three days. Gas sampling for CO_2 and N_2O analysis was carried out every 24 hours. As before, incubation vessels were flushed for 20 minutes after each sampling period.

Following incubation, soil cores were drained to -50 kPa matric potential, and as for the soil core incubations from the Galicos River Wastewater Treatment Project, soil cores were irrigated with another 20 ml of glucose and nitrogen solution and incubated for three days with flushing between sampling times. The head space was sampled for CO_2 and N_2O analysis every 24 hours.

Following incubation, the soils were removed from the cores and left to air dry at room temperature for three days. The soils were then sieved (2 mm-mesh size) and analyzed for electrical conductivity (EC), pH and particle size distribution. These data are presented in Tables A4.1 and A4.2.

Table A4.1 Characteristics of soil irrigated with wastewater (Q1), biologically treated water (Q2) or well water irrigation (Q3) at the Galicos River Wastewater Treatment Project in Greece. Values represent averages (n=4).

Parameter	(Q1) Soil irrigated with wastewater	(Q2) Soil irrigated with Biologically treated	(Q3) Soil irrigated with Well water
EC (dS m ⁻¹)	0.74	0.70	0.53
pH	8.8	8.7	8.3
Sand %	28	36	41
Silt %	47.5	46	43
Clay %	24.5	18	16

Table A4.2 Soil characteristics at the Axios Delta fields irrigated by flooding. Figures represent averages (n=2).

Parameter	Field-1	Field-2	Field-3	Field-4	Field-5	Field-6
EC (dS m ⁻¹)	6.20	0.50	0.42	0.40	0.60	0.58
PH	7.9	8.1	8	8.2	8.0	7.9
Sand %	8	7.5	1.50	5	41.1	64.2
Silt %	36.4	42	26	29.5	31.4	22.4
Clay	55.6	50.5	72.5	65.5	27.5	13.4

A4.3 Results and discussion

Soil cores that were irrigated by flooding did seem to have had a higher moisture content over a range of water tensions from -35 to -236 kPa (as shown in Figure

A4.1d) compared with soil cores irrigated using a furrow (Figure A4.1a, b, c). However, soil cores irrigated with well water using a furrow had the lowest moisture contents over a higher range of water potentials from - 232 kPa to - 618 kPa (Figure A4.1c).

There was variability in the soil water potential of cores at field moisture content, as measured using the filter paper technique (Figure A4.1a, b, c, d). These differences were observed between the soil cores irrigated either with the same or different water qualities. This might be due to the differences in soil texture and in particular, clay contents (Tables A4.1, A4.2). Another possibility is that it is due to the differences in their bulk density under different irrigation systems. The water retention curve of a soil can be altered with changes in the bulk density (Box and Taylor, 1962). The variability in soil water potential among soil cores appeared greatest under furrow irrigation using well water (Figure A4.1c) and represented a higher water potential from -232 to - 618.6 kPa. However, although the differences might be based on soil texture, gravimetric soil water content of different irrigated soils at -230 kPa was unaffected in most of these irrigated soils (Figure A4.1b, c, d). The lower soil water potentials were observed in soil cores under furrow irrigation using wastewater (Figure A4.1a). This might be due to the greater organic matter content of wastewater compared with the other irrigation sources (Table A4.1). As a result, water retention of soils increased. Burns and Rawitz (1981) observed that water retention was increased under effluent irrigation. Organic matter (OM) increases the water holding capacity (WHC) of soil (Papendick and Campbell, 1981) and, consequently, the actual distribution of water will be influenced by the distribution of OM. Matric potential is logarithmically related to gravimetric soil water content with the relationship depending mainly upon soil texture and organic matter content (Cassman and Munns, 1980). The water content in soil cores irrigated either with wastewater or flooding (Figure A4.1a, d) decreased less steeply with increasing matric potential under irrigation with biologically treated wastewater or well water (Figure A4.1b, c). This is good evidence for the effect of soil texture and particularly clay content on water retention in these soils under study.

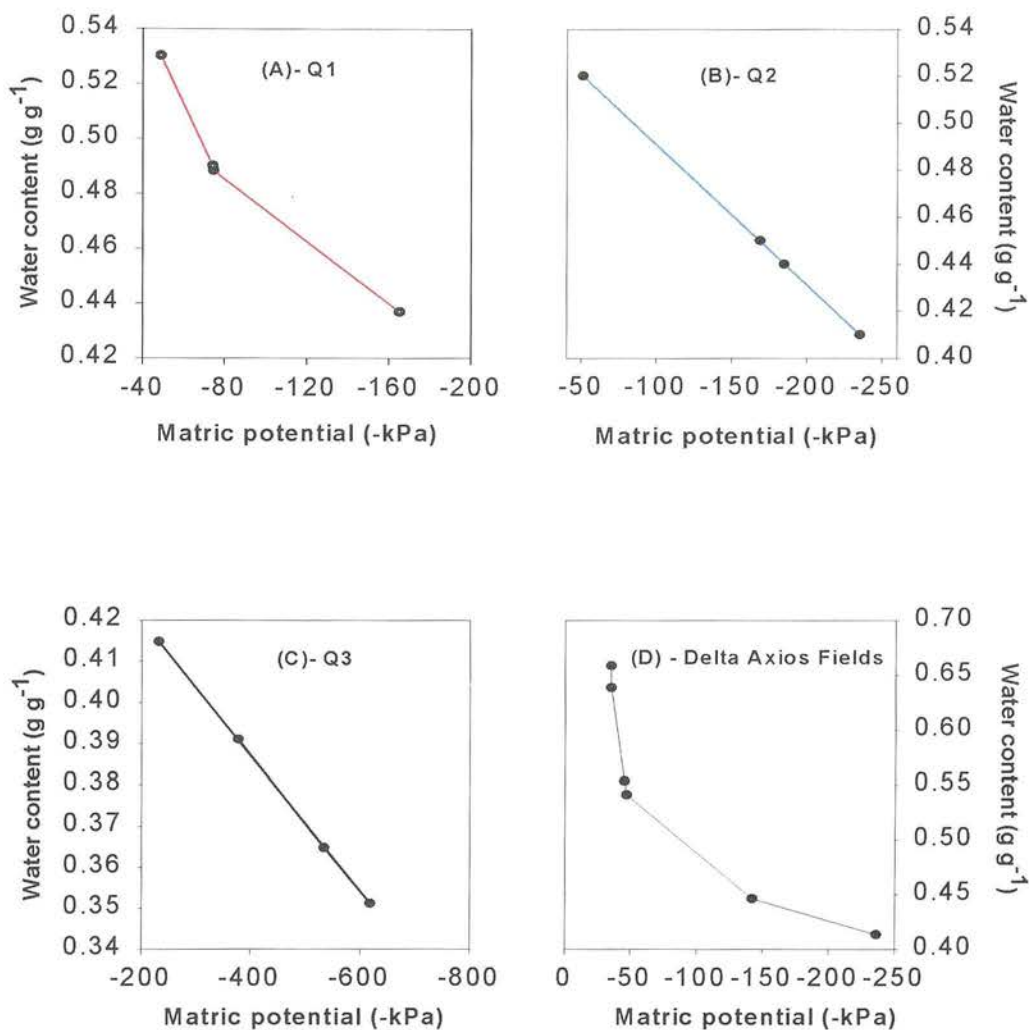


Figure A4.1 Soil water matric potential measured using a filter paper technique on intact soil cores irrigated either by a furrow using; (a) wastewater; (b) biologically treated wastewater; (c) well water; or by flooding (d), versus their field moisture content. Each point represents the measured soil water matric potential of a single soil core corresponding to its soil water content.

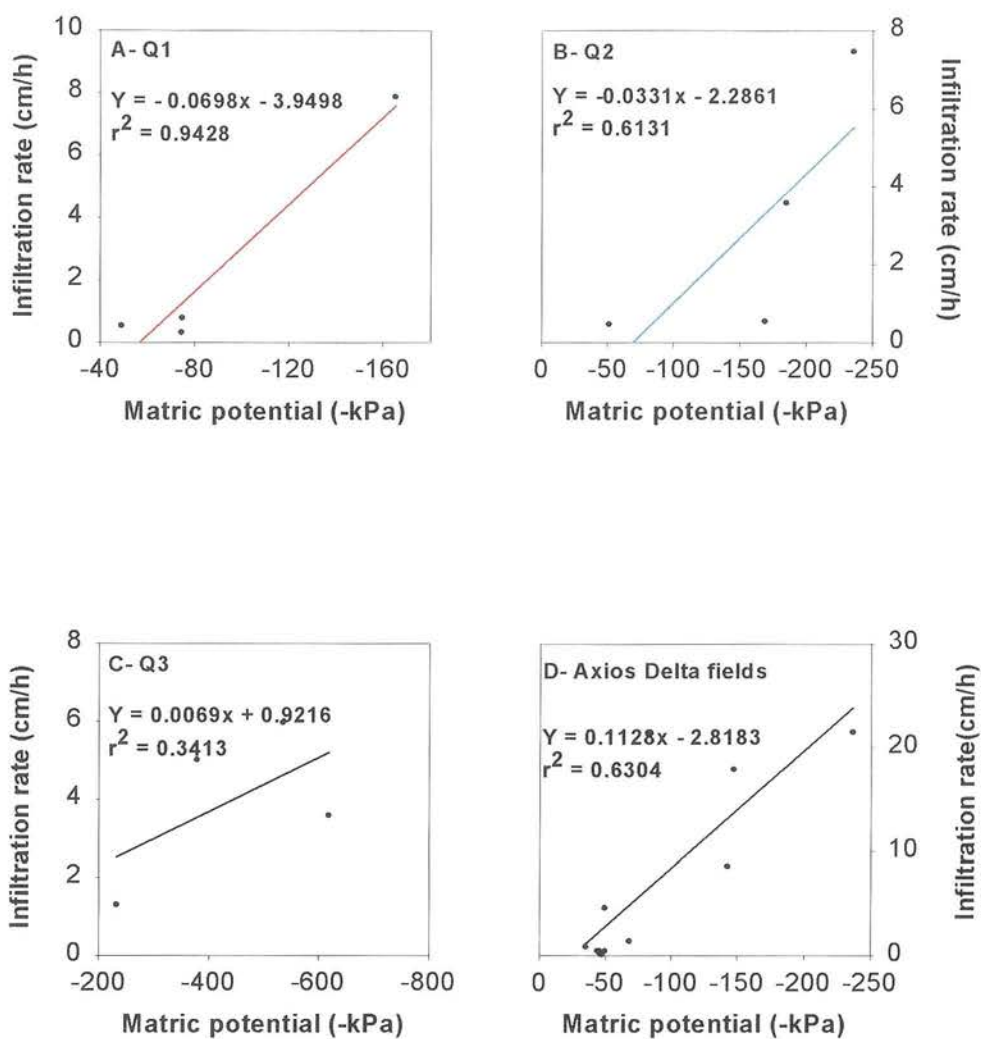


Figure A4.2 Soil water matric potential measured using a filter paper technique of intact soil cores irrigated either by a furrow using; (a) wastewater; (b) biologically treated wastewater; (c) well water; or by flooding (d), versus infiltration rate at their field moisture content.

Figure A4.2 represents the relationship between matric potentials (measured using the filter paper technique) and infiltration rates of intact soil cores at their field moisture contents. This figure shows that all these relationships are relatively strongly positive ($+ 0.34 < r < + 0.94$) (Figure A4.2a, b, c, d). Infiltration rates observed in soil cores from the Axios Delta fields irrigated using flooding were higher than those in soil cores from the Galicos River Wastewater Treatment Project cores irrigated using a furrow.

Figure A4.3 shows that the highest N_2O production rates of $189.9 \mu\text{g N kg}^{-1} \text{ dry soil day}^{-1}$ were measured in soil core incubations irrigated by furrow using well water (Q3), compared with maximum N_2O production rates of 37.6 and $26.9 \mu\text{g N kg}^{-1} \text{ dry soil day}^{-1}$ measured from soils irrigated with wastewater (Q1), or biologically treated wastewater (Q2), respectively (Figure A4.3a). This might be due to the further reduction of N_2O to N_2 under more anaerobic conditions in the presence of wastewater or biologically treated wastewater irrigation in the absence of acetylene inhibition. This is because of the high BOD_5 of wastewater, and also because the high sodium concentration in wastewater has the ability to disperse soil particles. As a result of the loss of structure, soil permeability problems occur, leading to poor aeration. The higher CO_2 evolution rates were not correlated with the higher N_2O -production rates (Figure 3 a, b). N_2O production rates in the same soil cores declined at a soil water potential -200 kPa (Figure A4.4 a), where the maximum N_2O -production rates of 13.7, 11.6 and $12 \mu\text{g N kg}^{-1} \text{ dry soil day}^{-1}$ were observed 12 hours after the beginning of the incubation after irrigation with either wastewater (Q1), biologically treated wastewater (Q2) or well water (Q3), respectively. This might be because soil moisture content influences denitrification directly by providing an environment suitable for micro-organisms through its effect on nutrient availability, O_2 tension, and microbial movement (Rolston, 1981).

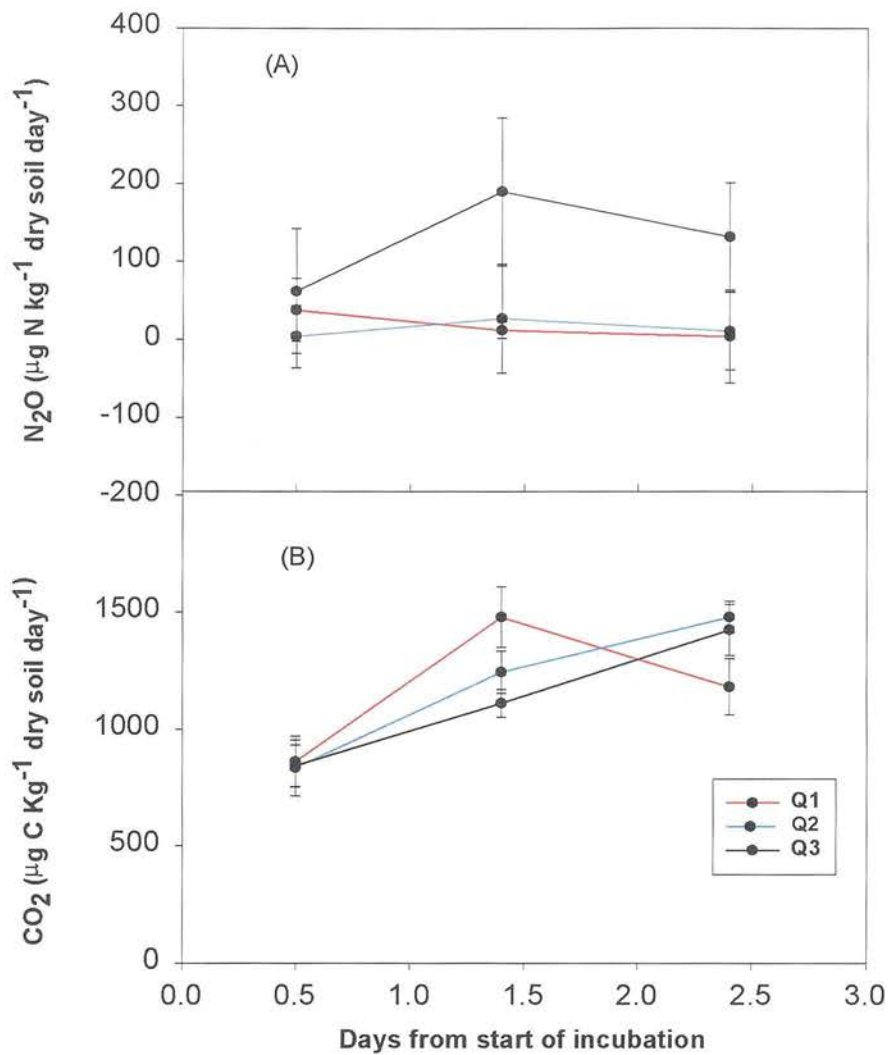


Figure A4.3 (A) N₂O-production and (B) CO₂-evolution rates of incubated intact soil cores, furrow-irrigated with either; (1) wastewater (Q1); (2) biologically treated wastewater (Q2); or (3) well water (Q3), in the field and with 20 of glucose and nitrogen solution. Each error bar represents the standard deviation of the respective mean of four replicates.

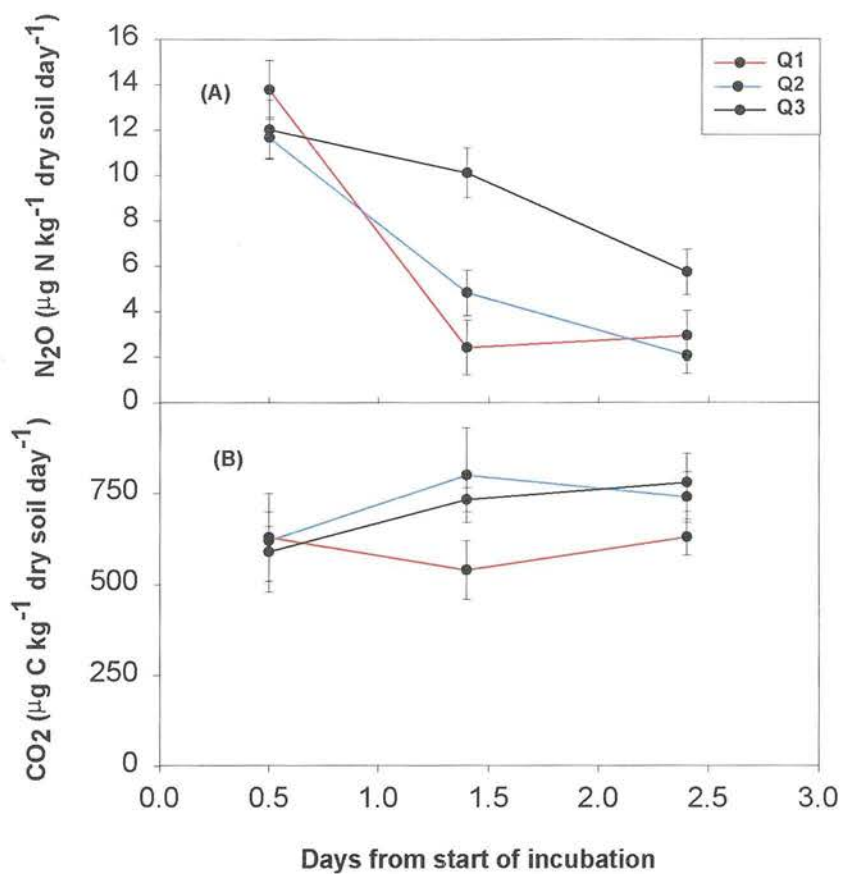


Figure A4.4 (A) N₂O production and (B) CO₂ evolution rates of the incubations of soil cores irrigated either with; (1) wastewater (Q1); (2) biologically treated wastewater; (Q2) or (3) well water (Q3), at -200 kPa soil water potential. Each error bar represents the standard deviation of the respective mean of four replicates.

After addition of 20 ml of glucose and nitrogen solution, good correlations ($r = -0.95$, -0.66) were observed between N_2O production rates of soil cores irrigated by furrow using either biologically treated wastewater (Q2) or well water (Q3), respectively, and soil matric potential (Figure A4.5 b, c). However, the correlation was low ($r = -0.23$) under wastewater irrigation (Figure A4.5a). This indicates that factors other than soil moisture were more important limitations to denitrification in wastewater-irrigated soil core incubations.

The mean denitrification rates of 18.8, 21.89, 58.10, 44.7, 15.7 and 1090 $\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day^{-1} were observed in soil cores from field No.s 1, 2, 3, 4, 5, and 6 at the Axios Delta, respectively (Figure A4.6a). However, the mean denitrification rates measured in the same soil cores at -50 kPa soil water potential were 11.88, 9.20, 47.7, 28.7, 10.3 and 264.3 $\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day^{-1} , respectively (Figure A4.7a).

The maximum N_2O production rates of 1200 $\mu\text{g N kg}^{-1}$ dry soil day^{-1} measured in soil cores irrigated using flooding were observed in soil cores from field No.6 which had a higher sand percentage (mean 64.20 %) and a lower clay content (mean 13.2 %) compared with those observed in soil cores from the other fields that had lower sand and higher clay content (Figure A4.6a, Table A4.2). This might be due to the faster diffusion of N_2O out of these coarser soils. Previous studies by Arah *et al.* (1991) have shown that N_2O fluxes measured from a loam soil profile were higher than those from clay loam soil plots supporting these results. Another possible explanation is that further reduction of N_2O to N_2 occurred in the soil cores from the other fields that had a higher clay content. The higher CO_2 evolution rates were not correlated with the highest N_2O production rates (Figure A4.6a, b, Figure A4.7a, b).

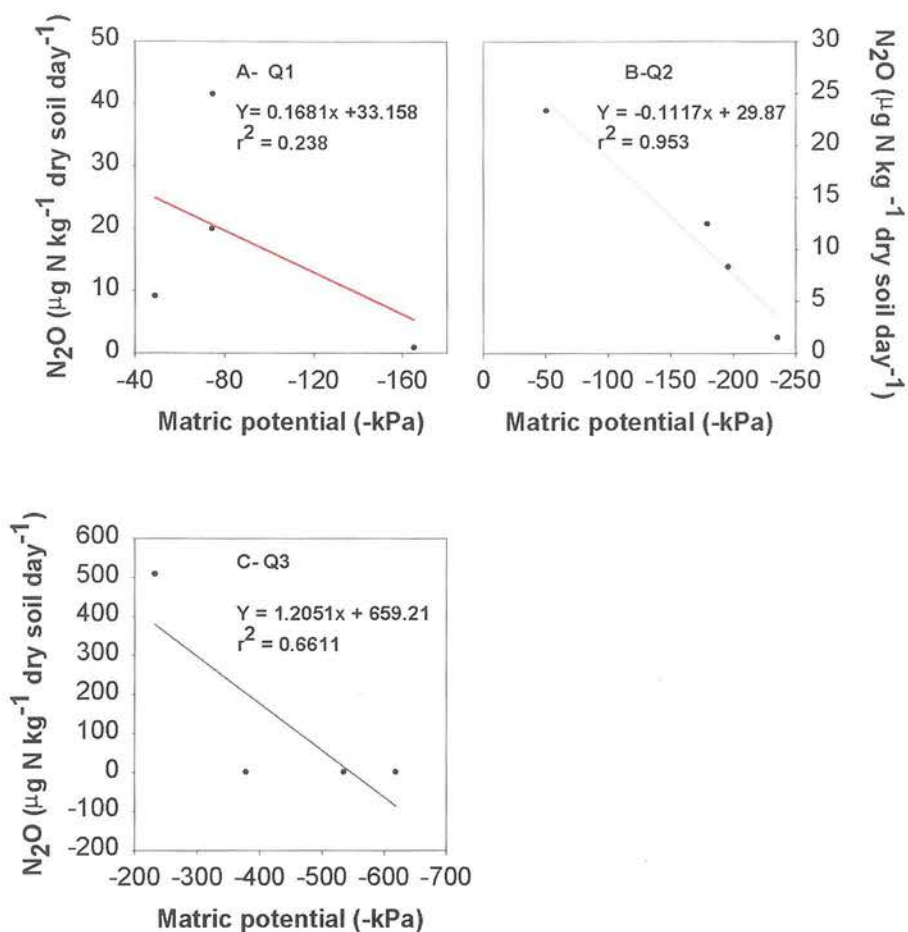


Figure A4.5 N₂O emission rates of intact soil cores irrigated either with; (a) wastewater; (b) biologically treated wastewater; or (c) well water in the field and with 20 ml of glucose and nitrogen fertilizer solution added, versus matric potentials measured at their field moisture content using a filter paper technique.

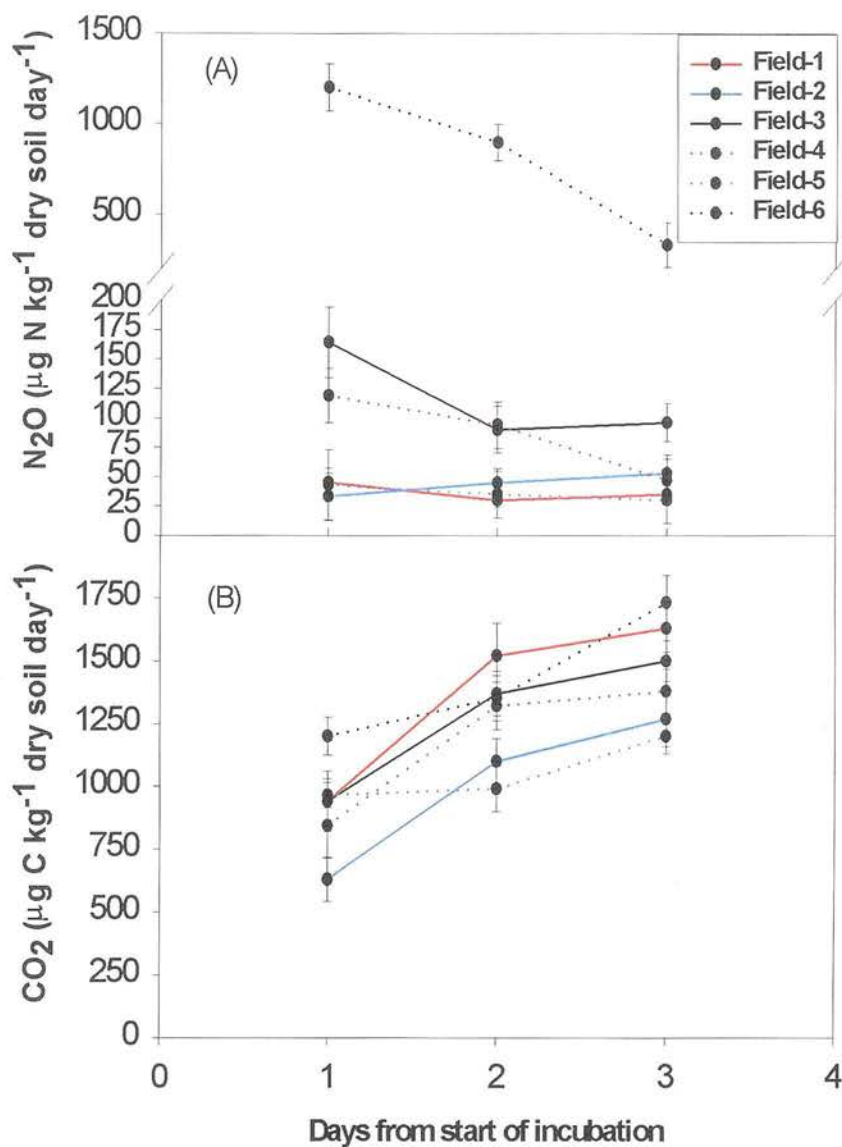


Figure A4.6 (A) N₂O production and (B) CO₂ evolution rates of soil core incubations from the Axios Delta fields irrigated by flooding after addition of 20 ml of glucose and nitrogen solution. Each error bar represents the standard deviation of the respective mean of two replicates.

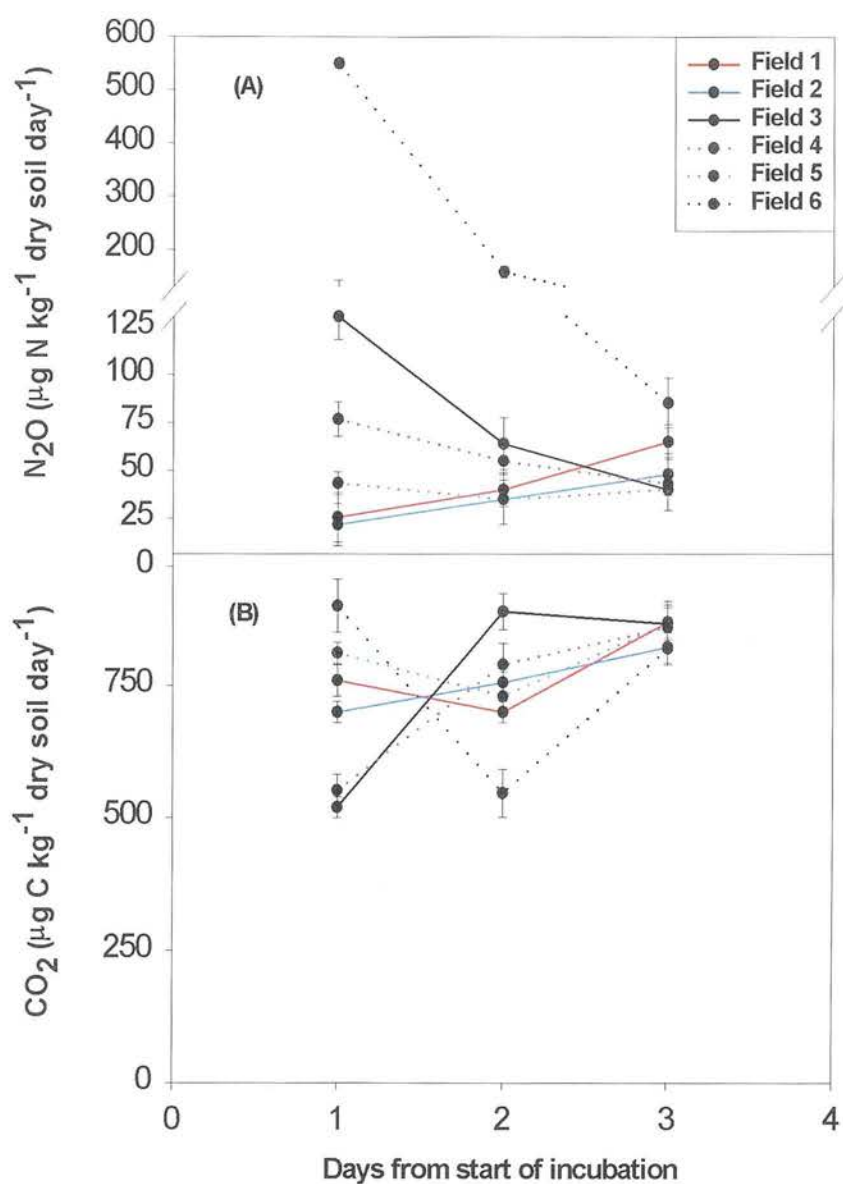


Figure A4.7 (A) N_2O production and (B) CO_2 evolution rates of soil core incubations from the Axios Delta fields irrigated by flooding at -50 kPa soil water potential. Each error bar represents the standard deviation of the respective mean of two replicates.

A4.4 Conclusion

Results from this experiment indicated that the microbial activity in different irrigated soils depends on soil water potential and soil texture especially clay content, since clay content is the main determinant of water availability to microorganisms, and it varied widely among the soils in this study.